PARAMENINGOCOCCUS AND ITS ANTISERUM.*

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In the course of the treatment of epidemic meningitis with the antimeningococcic serum it became evident that a proportion of cases of the disease treated with the serum failed to react favorably. In some of these cases the meningococcus contained in the cerebrospinal exudate survived, continued to multiply, and failed to be phagocyted by the leucocytes, in contradistinction to what happened in the majority of instances. That the meningococci occurring in the resistant cases were in part resistant, or fast, to the antiserum was suspected, and two possible kinds of fast strains were recognized: first, strains originally fast at the time the serum treatment was begun; and, second, strains developing fastness in the course of the serum treatment.[1] The second group embraced those instances in which meningococci at first reacted to the serum but later failed to do so, leading to a relapse which continued to a fatal termination.

A more precise definition of the fast strains has not thus far been made. However, Dopter[2] has studied a special class of the cocci obtained from the nasal secretion and called by him parameningococci, which while resembling the common meningococcus in fermentative and cultural properties differs from it in certain immunological reactions. The parameningococcus, so called, has now been found by Dopter and other French writers to invade the meninges and the blood and is believed by them to be one of the causes of cerebrospinal meningitis.[3] According to the French ob-

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[1] Flexner, S., *Jour. Exper. Med.*, 1913, xvii, 553.

[2] Dopter, Ch., *Compt. rend. Soc. de biol.*, 1909, lxvii, 74.

[3] The literature has been reviewed by Dr. Flexner in his article on antimeningococcic serum (Flexner, S., in Kraus, R., and Levaditi, C., Handbuch der Technik und Methodik der Immunitätsforschung, 2d edition, Jena, 1914 (in press)).

servers, when acute meningitis arises through the presence of the parameningococcus it is not controllable by means of the ordinary antimeningococcic serum, but does respond to a special antiserum prepared with cultures of the parameningococcus.

The subject of the atypical strains of the meningococcus is an important one since it affects the question of a better control of cases of epidemic meningitis by means of a serum containing antibodies for the unusual varieties. On that account we have made a study of two cultures of the parameningococcus obtained from Dr. Dopter through the kindness of Dr. Louise Pearce, who made a hurried journey to Paris to secure them, since it was found that the cultures sent by post did not survive the journey.

IMMUNITY REACTIONS OF THE MENINGOCOCCUS AND PARAMENINGOCOCCUS.

The meningococcus is subject to the several immunity reactions of agglutination, complement deviation, and opsonization. All the reactions are specific, although certain strains react not at all or imperfectly. As regards agglutination, it may be said that considerable variations arise affecting strains of meningococci relatively or absolutely inagglutinable. And yet agglutination is perhaps the most trustworthy guide in the identification of the meningococcus through specific serum reactions.[4] While immune sera may possess high agglutinating value for meningococci, normal sera possess either none, or very little; the inagglutinable strains react to immune sera of high value no more than they do to normal sera.

On the one hand, the inagglutinable strains react differently to a given meningococcic serum, while again a given agglutinable strain may react unequally to immune sera prepared from different strains of meningococci.[5] It should further be noted that while homologous sera tend to agglutinate corresponding strains best, exceptions occur in which they agglutinate heterologous strains better.[5,6]

[4] Flexner, S., in Kraus, R., and Levaditi, C., Handbuch der Technik und Methodik der Immunitätsforschung, *loc. cit.* Elser, W. J., and Huntoon, F. M., *Jour. Med. Research,* 1909, xx, 371.

[5] Eberle, J., *Arch. f. Hyg.,* 1908, lxiv, 171.

[6] St. Baecher, in Kraus, R., and Levaditi, C., Handbuch der Technik und Methodik der Immunitätsforschung, Jena, 1911, supplement 1, 80.

Notwithstanding the variations indicated, it is obviously still desirable to determine the degree in which parameningococci differ from normal meningococci in respect to immunity reactions, and to ascertain whether they form a special class or merely constitute variations from the normal type. For this purpose two cultures, L and M, of the parameningococcus secured from Dr. Dopter have been subjected to the reactions of agglutination, complement deviation, and opsonization, respecting which they have been compared with several strains of normal meningococci. The immune sera employed consisted of a polyvalent antimeningococcic serum prepared by the Department of Health of the City of New York, and several monovalent sera produced in the rabbit by immunization with single strains of the microörganisms.

It will conduce to clearness and simplicity of presentation to describe briefly the different strains of meningococci which were studied.

The parameningococci consisted of Gram-negative diplococci indistinguishable from ordinary meningococci in form, staining properties, and fermentative reactions. They were also subject to autolysis in the manner of normal meningococci.

Twenty other strains of meningococci were employed for comparison. Four, HP, MA, B, and 138, came from the Pasteur Institute, having been secured by Dr. Pearce along with the parameningococci. They were regarded as normal strains. It may be noted here that all four exhibited irregularities of agglutination, and B proved inagglutinable.

One strain, W, came from Great Britain in 1908, and was isolated from a case of posterior basic meningitis. It was a normal strain. The remaining fifteen strains were obtained in New York, partly from the stock of The Rockefeller Institute, partly from the Department of Health.[7] They have been classified as follows: Eight are normal strains agglutinating regularly. They are designated F, 20, 25, 28, 35, 45, 48, and 49. Cultures 25 and 48 were derived from rapidly fatal fulminating cases of meningitis; 28 is from a severe case terminating fatally on the fourth day; 45 is from a fatal case developing basic symptoms; 35 is from a mild case becoming chronic, in which hydrocephalus developed before death; and 49 is from a mild case which recovered.

Five are normal strains agglutinating irregularly. BH was derived from a fatal case in an infant; 9 and 18 were without history; and 37 was obtained from a case which recovered under serum treatment. Two strains, 7 and 42, gave agglutination reactions similar to the parameningococci, and had been employed in the manufacture of the antimeningococcic serum by the New York Department of Health. Table I furnishes a means of ready reference.

[7] For the Board of Health cultures I am indebted to Dr. Phoebe Du Bois and Dr. Marie Grund.

TABLE I.

Source of Cultures.

Designation.	Source.	Nature of strain.	Type of agglutination.
L	Dopter	Para	
M	Dopter	Para	
HP	Pasteur Institute	Normal	Irregular.
MA	Pasteur Institute	Normal	Irregular.
138	Pasteur Institute	Normal	Irregular.
B	Pasteur Institute	Normal	Inagglutinable.
W	Great Britain	Normal	Regular.
F	New York	Normal	Regular.
18	New York	Normal	Regular.
25	New York	Normal	Regular.
28	New York	Normal	Regular.
35	New York	Normal	Regular.
45	New York	Normal	Regular.
48	New York	Normal	Regular.
49	New York	Normal	Regular.
BH	New York	Normal	Irregular.
I	New York	Normal	Irregular.
9	New York	Normal	Irregular.
20	New York	Normal	Irregular.
37	New York	Normal	Irregular.
7	New York	Normal	Para-like.
42	New York	Normal	Para-like.

Besides the results of agglutination, other immunity reactions were studied; those, namely, of opsonization, complement deviation, and protection. These reactions are subject also to irregularities and variations, and notably that of complement deviation,[8] which has been generally given up as a method of determining the value of the antimeningococcic serum in therapeutic immunity principles.

AGGLUTINATION.

Several immune sera were employed for determining the immunity reactions. One, that of the New York Health Department, was prepared in the horse from many strains of meningococcus and preserved with 0.3 per cent. tricresol. The monovalent sera were made by immunizing rabbits with single strains. The inoculations were conducted over periods of several months. For the general

[8] Flexner, S., in Kraus, R., and Levaditi, C., Handbuch der Technik und Methodik der Immunitätsforschung, *loc. cit.*

TABLE II.

Monovalent Rabbit Serum Immune to Parameningococcus L. Agglutination Reactions Made at 55° C.

Strains.	C	10	20	50	100	200	500
Para L	−	++	++	++	++	++	+
Para M	−	++	++	+	+	+	−
48	−	+	+	±	−	−	−
45	−	+	−	−	−	−	−
49	−	++	+	±	−	−	−
42	−	++	++	++	+	+	−
35	−	++	−	−	−	−	−
37	−	+	+	+	+	−	−
138	−	+	+	±	−	−	−
28	−	++	±	−	−	−	−
25	−	++	++	+	±	−	−
20	−	++	++	++	+	±	−
18	−	++	++	++	+	±	−
9	−	++	++	+	±	−	−
7	−	++	++	+	+	−	−
B	−	−	−	−	−	−	−
MA	−	+	+	+	−	−	−
HP	−	+	+	+	−	−	−
W	−	++	+	+	±	−	−
I	−	+	+	+	−	−	−
BH	−	+	+	+	+	−	−
F	−	+	+	−	−	−	−

TABLE III.

Monovalent Rabbit Serum Immune to Parameningococcus M. Agglutination Reactions Made at 55° C.

Strains.	C	10	20	50	100	200	500
Para M	−	++	++	++	++	+	+
Para L	−	++	++	+	+	±	−
48	−	+	±	−	−	−	−
45	−	+	+	+	−	−	−
49	−	++	++	+	±	−	−
42	−	++	++	++	+	+	−
35	−	+	±	−	−	−	−
37	−	++	++	+	±	−	−
138	−	+	+	+	−	−	−
28	−	+	+	−	−	−	−
25	−	+	+	±	−	−	−
20	−	++	++	++	+	−	−
18	−	++	++	++	+	−	−
9	−	++	++	++	+	−	−
7	−	++	++	++	+	+	−
B	−	+	−	−	−	−	−
MA	−	+	+	+	+	±	−
HP	−	+	+	+	+	+	−
W	−	++	+	±	−	−	−
I	−	++	++	++	+	+	−
BH	−	+	+	+	+	+	−
F	−	+	+	±	−	−	−

work the two Dopter strains of parameningococci and normal strains 35 (mild case), 45 (basic case), and 48 (fulminating case) were used.

At the expiration of three months the titer of the rabbit sera did not exceed 1 to 500. But as normal rabbit serum is inactive in dilutions greater than 1 to 10, the specific effects could be readily followed. A few exceptions with normal strains occurred. Thus strains 45 and 138 agglutinated in 1 to 20, and strain 37 in 1 to 50. Normal horse serum is inactive above 1 to 20. Here again certain normal strains, namely 25, 45, and 138, were somewhat more sensitive and reacted in 1 to 50 to 1 to 100 dilutions.

Parameningococcus.—The polyvalent antimeningococcic horse serum was almost wholly inactive upon the two Dopter strains of parameningococci, while it agglutinated the two para-like strains 7 and 42 in dilutions 1 to 200. It should be recalled that the two latter strains were employed in the preparation of the serum.

The monovalent parameningococcus rabbit sera exhibited varying titers according as they acted upon the homologous or heterologous strains. With the former the limit was 1 to 500, with the latter 1 to 200. No difference was noted between the sera prepared from strains L or M (tables II and III).

On the other hand, the two para-like strains, 7 and 42, gave slightly different reactions according to the source of the immune parameningococcus serum. With para serum L, strain 42 agglutinated at 1 to 200, and strain 7 at 1 to 100; with para serum M, both agglutinated at 1 to 200. In other words, strains 7 and 42 behave as heterologous para strains against these two sera.

If we turn now to a monovalent serum prepared from normal strain 48 which agglutinated its own and two other normal strains in 1 to 500, the two Dopter para strains, L and M, agglutinated in 1 to 20, and the two para-like strains at 1 to 20 (strain 42) and 1 to 50 (strain 7) (table IV).

However, the Dopter para sera are not without agglutinating effects on normal strains of meningococcus, and both those that agglutinate regularly and irregularly. With para serum L, among the former, strain 18 reacts in 1 to 100, strains 25 and W in 1 to 50 dilutions; among the latter, strains 37 and BH react in 1 to 100,

strain 20 in 1 to 100 dilutions. With para serum M regular strains 18 and 49 react in dilutions of 1 to 100 and 1 to 50, respectively, irregular strains HP, I, and BH react in 1 to 200, and strains 9 and 20 in 1 to 100 dilutions.

TABLE IV.

Monovalent Rabbit Serum Immune to Meningococcus 48. Agglutination Reactions Made at 55° C.

Strains.	C	10	20	50	100	200	500
48	−	++	++	++	++	+	+
Para M	−	++	+	±	−	−	−
Para L	−	++	+	−	−	−	−
45	−	+	+	+	+	−	−
42	−	+	+	−	−	−	−
49	−	++	++	++	+	−	−
35	−	++	++	++	++	+	+
37	±	++	++	++	++	+	−
138	−	++	++	++	++	+	−
28	−	++	++	+	−	−	−
25	−	++	++	++	++	++	+
20	−	++	+	+	−	−	−
18	−	++	++	++	++	+	±
9	−	++	++	+	±	−	−
7	−	++	++	±	−	−	−
B	−	+	+	±	−	−	−
MA	−	+	+	+	+	−	−
HP	−	+	+	+	+	+	−
W	−	++	++	++	+	±	−
I	−	++	+	+	±	−	−
BH	−	++	+	+	+	+	−
F	−	++	++	++	+	−	−

The conclusion to be drawn from this series of tests is not to the effect that parameningococcus strains are strictly different as regards agglutination from normal strains of meningococcus, but that they nevertheless display a certain relative specificity.

Normal Meningococcus.—Two classes of normal meningococci have been recognized. They have been denominated "regular" and "irregular" according as they agglutinate in all or only in part of the normal immune sera. The variations in regard to agglutinability among normal strains are wide, as is exhibited in table V. It is this great variability that makes it impracticable on the basis of agglutination alone to separate certain strains as being a distinct group or species. It remains true, however, that the group distinguished by the name of "para" departs even more widely from the normal

standard than do the several irregular strains studied. And this difference reappears in respect to other immune reactions to be described.

TABLE V.

Agglutination Reaction at 55° C. Limit Dilutions for Complete Agglutination.

Strain.	Sera.				
	Board of Health.	Para M.	Para L.	Normal 48.	Normal 35.
Para L	1 : 10	1 : 100	1 : 500	1 : 20	1 : 10
Para M	1 : 10	1 : 500	1 : 200	1 : 20	1 : 20
Para-like 7	1 : 200	1 : 200	1 : 100	1 : 20	1 : 50
Para-like 42	1 : 100	1 : 200	1 : 200	1 : 20	1 : 50
Normal regular W	1 : 500	1 : 20	1 : 50	1 : 100	1 : 100
Normal regular F	1 : 50	1 : 20	1 : 20	1 : 100	1 : 50
Normal regular 18	1 : 50	1 : 100	1 : 100	1 : 200	1 : 100
Normal regular 25	1 : 50	1 : 20	1 : 50	1 : 500	1 : 200
Normal regular 28	1 : 100	1 : 20	1 : 10	1 : 50	1 : 50
Normal regular 35	1 : 10	1 : 10	1 : 10	1 : 500	1 : 500
Normal regular 45	1 : 50	1 : 50	1 : 10	1 : 100	1 : 200
Normal regular 48	1 : 50	1 : 10	1 : 20	1 : 500	1 : 50
Normal regular 49	1 : 100	1 : 50	1 : 20	1 : 100	1 : 100
Normal irregular BH	1 : 500	1 : 200	1 : 100	1 : 200	1 : 200
Normal irregular I	1 : 100	1 : 200	1 : 50	1 : 50	1 : 100
Normal irregular 9	1 : 200	1 : 100	1 : 50	1 : 50	1 : 50
Normal irregular 20	1 : 50	1 : 100	1 : 100	1 : 50	1 : 100
Normal irregular 37	1 : 100	1 : 50	1 : 100	1 : 200	1 : 100
Normal irregular 138	1 : 200	1 : 50	1 : 20	1 : 200	1 : 50
Normal irregular HP	1 : 20	1 : 200	1 : 50	1 : 200	1 : 200
Normal irregular MA	1 : 200	1 : 100	1 : 50	1 : 100	1 : 100
Inagglutinable B	1 : 20	1 : 10	0	1 : 20	0

Hence it is apparent that a clean cut classification into parameningococcus and meningococcus strains has been possible with thirteen only of the twenty-two strains whose agglutination reactions have been studied. The remaining nine act either irregularly or so nearly alike in all the sera tested that definite discrimination is not possible.

Attention is directed also to the fact that lack of agglutination by polyvalent antimeningococcic horse serum is insufficient evidence for the classification of meningococci into para and normal strains, since even normal meningococci do not invariably agglutinate in such a serum in high dilution, and some strains fail to agglutinate in dilutions greater than 1 to 20.

OPSONINS AND COMPLEMENT DEVIATION.

Opsonins.—The opsonin content is employed extensively for determining the therapeutic value of antimeningococcic serum. It was desirable therefore to test the specificity of this reaction upon normal and para strains of meningococci. For this purpose the Neufeld technique was employed. The result is shown in table VI, which tends again to isolate the two parameningococcus strains of Dopter from the strains of normal meningococcus employed.

TABLE VI.

Opsonization.

Serum.	Strain.	Control.	1:10	1:20	1:50	1:100	1:200	1:500	1:1,000	1:2,000	1:5,000
Board of Health	Para L	−	+	−	−	−	−	−	−	−	−
	Para M	−	+	−	−	−	−	−	−	−	−
	48	−	+	++	+	+	+	±	−	−	−
	35	±	++	+	+	±	−	−	−	−	−
	I	−	++	++	++	++	+	+	+	+	±
Fulminating case	48	−	+	+	+	+	+	±	−	−	−
	35	−	++	++	++	+	+	±	−	−	−
	Para L	−	+	+	+	−	−	−	−	−	−
	Para M	−	+	+	−	−	−	−	−	−	−
	I	−	+	+	+	±	−	−	−	−	−
Para M	Para M	−	+	+	+	+	+	+	−	−	−
	Para L	−	+	+	+	+	−	−	−	−	−
	48	−	+	+	±	−	−	−	−	−	−
	I	−	+	+	±	−	−	−	−	−	−
Para L	Para L	−	+	+	+	+	+	±	−	−	−
	Para M	−	+	+	+	+	−	−	−	−	−
	48	−	+	+	+	−	−	−	−	−	−
	I	−	+	±	−	−	−	−	−	−	−
Mild case	35	−	++	++	++	++	+	±	−	−	−
	48	−	++	++	+	+	±	−	−	−	−
	Para L	−	+	+	−	−	−	−	−	−	−
	Para M	−	+	+	−	−	−	−	−	−	−

Complement Deviation.—The degree of deviation of complement exerted by an antimeningococcic serum was recommended by Kolle and Wassermann to estimate its therapeutic value. Since, however, it appears that the reaction is subject to considerable and unexplained fluctuations it has not been generally adopted. None the less, it was desirable to determine the degree of specificity of the reaction as applied to para and normal strains.

Antigens were prepared by the method of Schwartz and McNeil.[9] When the antigens made with the parameningococci of Dopter were tested against the polyvalent antimeningococcic horse serum, complement was bound. When, however, the monovalent parameningococcic sera were titrated against normal meningococci, complement deviation occurred only in low dilutions. When the parameningococci antigens were titrated against monovalent sera prepared with normal meningococci strains 48 and 35, no binding was obtained. On the other hand, antigens of strains 48 and 35 deviated complement of the homologous sera in low dilutions (table VII). The action of the polyvalent antimeningococci serum corresponds with the results obtained by Dopter,[10] who, however, also noted that the sera of patients suffering from parameningococcal infection deviated complement in the presence of parameningococci but not of normal meningococci. If this is strictly true, then it must be held that the monovalent rabbit sera are less specific than the serum of patients.

The conclusion to be drawn from the tests of complement deviation is again to the effect that the para strains deviate from the normal strains, although the distinction cannot be said to be absolute.

PROTECTION EXPERIMENTS.

In the end the existence of profound differences between the para and normal strains of meningococci will be determined not so much by the immunity reactions already described, as by the results of protection tests, since in practice it is this test that determines whether special account should be taken of the para strains in the preparation of the antimeningococcic serum. For the purpose of the protection tests guinea pigs and monkeys were employed.

That guinea pigs weighing about 125 grams are especially susceptible to inoculation with cultures of the meningococcus was noted by Flexner.[11] I am able to confirm his observation. The tests were conducted with lethal doses by intraperitoneal injection of the

[9] Schwartz, H. J., and McNeil, A., *Am. Jour. Med. Sc.*, 1912, cxliv, 815.
[10] Dopter, *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1911, xxxi, series 3, 590.
[11] Flexner, S., *Jour. Exper. Med.*, 1907, ix, 105.

TABLE VII.

Complement Deviation.

Complement, guinea pig serum, 1:40 dilution, in c.c.	Immune rabbit serum Para M, in c.c.	Para M antigen, in c.c.	Sheep corpuscles, 1:20 dilution, in c.c.	Anti-sheep rabbit serum, 1:100 dilution, in c.c.	Result.
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.2	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	No hemolysis.
		Culture 48 antigen.			
0.1	0.1	0.25	0.1	0.1	Incomplete hemolysis.
0.1	0.2	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	Complete hemolysis.
	Immune serum, fulminating case.				
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	No hemolysis.
0.1	0.01	0.25	0.1	0.1	No hemolysis.
		Culture 35 antigen.			
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	No hemolysis.
0.1	0.01	0.25	0.1	0.1	No hemolysis.
		Para L antigen.			
0.1	0.1	0.25	0.1	0.1	Complete hemolysis.
0.1	0.1	0.15	0.1	0.1	Complete hemolysis.
0.1	0.01	0.25	0.1	0.1	Complete hemolysis.
		Para M antigen.			
0.1	0.1	0.25	0.1	0.1	Complete hemolysis.
0.1	0.1	0.15	0.1	0.1	Complete hemolysis.
0.1	0.01	0.25	0.1	0.1	Complete hemolysis.
	Board of Health serum.				
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	Complete hemolysis.
0.1	0.01	0.25	0.1	0.1	Complete hemolysis.
		Para L antigen.			
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	No hemolysis.
0.1	0.01	0.25	0.1	0.1	Complete hemolysis.
		Culture 48 antigen.			
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	Incomplete hemolysis.
0.1	0.01	0.25	0.1	0.1	Incomplete hemolysis.
		Culture 35 antigen.			
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	No hemolysis.
0.1	0.01	0.25	0.1	0.1	Complete hemolysis.

several cultures alone and combined with homologous and heterologous sera. Preliminary experiments were made to determine the effects of normal rabbit and horse serum. They were found not to protect in corresponding doses against the cultures used. The cultures alone in the doses given invariably caused death. The following is a detailed example of a protective experiment in young guinea pigs, of which table VIII presents the results in brief.

Experiment 1.—A. May 27, 1914. Two guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M.

May 28. Both guinea pigs were dead.

B. May 27. Four guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M, followed at once by 0.5 c.c. of Para M immune rabbit serum.

May 28. Four guinea pigs were living but one was ill.

May 29. One guinea pig was dead.

C. May 27. Four guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M, followed at once by 0.5 c.c. of Para L immune rabbit serum.

May 28. All four guinea pigs were dead.

D. May 27. Four guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M, followed at once by 0.5 c.c. of immune rabbit serum 48.

May 29. Three guinea pigs were dead.

E. May 27. Four guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M, followed at once by 0.5 c.c. of immune rabbit serum 35.

May 28. All four guinea pigs were dead.

F. May 27. Four guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M, followed at once by 0.5 c.c. of Board of Health antimeningitis serum.

May 28. One guinea pig was dead.

What is noticeable is the general fluctuation of protection in that each monovalent serum, while being most perfectly protective for its homologous organism, exerts, also, some, if variable, amounts of protection against other or heterologous organisms. In this regard it cannot be said that the para strains of Dopter acted more regularly and specifically than the normal strains. On the whole, and as was probably to be expected, the Board of Health polyvalent serum showed the greatest regularity of action. In other words, this polyvalent serum carried protective immune bodies in about equal amount for the normal and para strains.

It was deemed desirable to ascertain the protective value of mono-

TABLE VIII.

Serum Protection Experiments.

Serum.	Cultures employed.				
	Para L.	Para M.	Culture 48.	Culture 35.	Culture 45.
Para L	Protected all	Protected none	Protected 8 of 10	Protected all	Protected none.
Para M	Protected 4 of 10	Protected 7 of 10	Protected 5 of 10	Protected none	Protected none.
Culture 48	Protected 6 of 10	Protected 4 of 10	Protected all	Protected 9 of 10	Protected none.
Culture 35	Protected 4 of 8	Protected none	Protected 5 of 6	Protected all	Protected none.
Board of Health	Protected 5 of 6	Protected 5 of 6	Protected 5 of 6	Protected 3 of 6	
Normal horse	Protected none	Protected none	Protected none	Protected none	
Normal rabbit	Protected none	Protected none	Protected none	Protected none	

valent sera upon monkeys infected by intraspinal inoculation and treated in the same manner. For this purpose parameningococcus L (Dopter) was employed for infection. The culture proved to be of low virulence, necessitating large doses in order to set up fatal infection. The method was to inject the culture and then immediately afterwards the immune sera. The following small series of experiments was made.

Experiment 1.—Control. A *Macacus rhesus* received intraspinally the surface growths of two sheep serum water agar slant cultures suspended in normal saline. Three hours after the injection the animal became ill; death occurred in twenty hours. At autopsy the meninges were congested and edematous; cultures of parameningococcus L were recovered.

Experiment 2.—A second *Macacus rhesus,* having received a similar dose of the suspended culture, was given five minutes later 1.5 c.c. of parameningococcus rabbit serum L. Slight symptoms of illness only developed. Twenty-four hours later lumbar puncture yielded turbid fluid containing polynuclear leucocytes enclosing diplococci; no diplococci were found outside of cells. A second dose of 1.5 c.c. of the immune serum was administered. At the expiration of another twenty-four hours the animal appeared well and the cerebrospinal fluid was clear.

Experiment 3.—A third *Macacus rhesus* was inoculated with the established dose of parameningococcus L and five minutes later was given 1.5 c.c. of immune rabbit serum prepared from normal meningococcus 48. No protection was afforded, and death occurred within twenty hours.

Experiment 4.—The fourth and last test was made with parameningococcus L and immune rabbit serum prepared with parameningococcus M. It was a repetition of experiment 2. The animal recovered completely.

The series of tests with monkeys is of value in supporting the group distinction between the normal and para meningococci. It is highly improbable that in a larger series of experiments some degree of cross-protection should not have been found between the normal and para organisms. On the other hand, the experiments indicate that para sera L and M are equally effective for protection against a given parameningococcus as in the case of para organism L.

DISCUSSION.

The study of *Diplococcus intracellularis* or meningococcus and allied organisms has led to the setting up of four classes as follows: (1) pseudomeningococci found by von Lingelsheim;[12] (2) diplococci derived from cases of posterior basilar meningitis described by Houston[13] and other English workers; (3) S strains isolated by Friese and Müller[14] from the nasopharynx of patients not having meningitis, and classified by Sachs-Müke[15] as pseudomeningococci; and finally (4) the diplococci described by Dopter as parameningococci. Von Lingelsheim's cocci are so readily differentiated from true meningococci by their morphological and cultural characteristics that they require no further mention. The other three classes, however, are described as being morphologically and culturally indistinguishable from true meningococcus, differing only in serum reactions, especial stress being laid upon differences in agglutinating power.

The diplococci from cases of basilar meningitis have been shown to be true meningococci,[16] a fact further substantiated by the tests

[12] von Lingelsheim, W., *Klin. Jahrb.*, 1906, xv, 373.
[13] Houston, T., and Rankin, J. C., *Brit. Med. Jour.*, 1907, ii, 1414.
[14] Friese, H., and Müller, H., *Klin. Jahrb.*, 1909, xx, 321.
[15] Sachs-Müke, *Klin. Jahrb.*, 1911, xxiv, 425.
[16] Wollstein, M., *Jour. Exper. Med.*, 1909, xi, 579.

made in the present study with diplococci from two personal cases of chronic basilar meningitis, and by one strain sent by Dr. Houston in 1908.

The S cocci described by Friese and Müller were not obtained from meningitis patients nor from persons who had been in contact with cases of meningitis, and all the cultures differed markedly in agglutination reactions from strains of true meningococcus. In the absence of other serum tests it is not possible to bring these cocci into relation with parameningococci.

Dopter's parameningococci remain, then, in a class by themselves, differing serologically more or less from other diplococci. Although Dopter first found them in the nasal mucus of persons who had been in contact with meningitis patients, other observers soon demonstrated their presence in the blood and cerebrospinal fluid. Thus in 1910 a case of purpura fulminans with septicemia was observed by Carnot and Marie,[17] from which the organism was isolated from the blood. No meningitis was present in this case. Menetrier[18] was the first to report a case of meningitis due to parameningococcus. The patient was an infant and Menetrier noted a marked difference between the apparent mildness of the symptoms and the intensity of the infection as evidenced by the character of the cerebrospinal fluid, in which the majority of the cocci were extracellular. Injections of the usual antimeningococcic serum instead of causing amelioration of symptoms and fall in temperature were followed by intensification of symptoms. Seven cases of meningitis due to the parameningococcus having come under Dopter's observation, he recommended the therapeutic use of ordinary antimeningococcic serum to be followed later by an antiparameningococcic serum if laboratory examination showed the presence of parameningococcus infection. Dopter[19] prepared such an antiparameningococcus serum in 1912, and its use was followed by the recovery of cases of meningitis caused by the parameningococcus

[17] Carnot and Marie, P.-L., *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1911, xxxi, series 3, 74.

[18] Menetrier, *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1911, xxxi, series 3, 82.

[19] Dopter, *Semaine méd.,* 1912, xxxii, 298.

(Salin and Reilly,[20] Mery, Salin, and Wilborts,[21] Menetrier and Avezou,[22] and Hallé[23]). In these cases two or three injections of ordinary antimeningococci serum were said to have been without effect, while the injection of the antiparameningococcus serum was followed by prompt improvement. Hallé, noting that only extracellular cocci were present in the cerebrospinal fluid after the ordinary serum had been given, did not wait for cultures of the diplococcus before resorting to the antiparameningococcus serum. He believes that a mixture of para and true meningococcus serum will give good results in the treatment of meningitis, though he agrees with Netter[24] that polyvalent serum is best. Netter uses a mixture of the two sera, but believes that a polyvalent serum, like that made in America, and which has given him excellent results, fulfills all requirements.

SUMMARY AND CONCLUSIONS.

The parameningococci of Dopter are culturally indistinguishable from true or normal meningococci, but serologically they exhibit differences as regards agglutination, opsonization, and complement deviation.

Because of the variations and irregularities of serum reactions existing among otherwise normal strains of meningococci it does not seem either possible or desirable to separate the parameningococci into a strictly definite class. It appears desirable to consider them as constituting a special strain among meningococci not, however, wholly consistent in itself.

The distinctions in serum reactions between normal and para meningococci are supported by the differences in protective effects of the monovalent immune sera upon infection in guinea pigs and monkeys.

[20] Salin, H., and Reilly, J., *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1913, xxxv, series 3, 423.

[21] Mery, H., Salin, H., and Wilborts, A., *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1913, xxxv, series 3, 411.

[22] Menetrier, P., and Avezou, J., *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1914, xxxvii, series 3, 45.

[23] Hallé, *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1914, xxxvii, series 3, 149.

[24] Netter, *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1914, xxxvii, series 3, 53.

It is therefore concluded that it is highly desirable to employ strains of parameningococcus in the preparation of the usual polyvalent antimeningococcic serum. It remains to be determined whether it is better to employ the parameningococci along with normal meningococci in immunizing horses, or to employ normal and para strains separately in the immunization process and to combine afterwards, in certain proportions, the sera from the two kinds of immunized horses.

A STUDY OF THE METABOLISM OF CALCIUM, MAGNESIUM, SULPHUR, PHOSPHORUS, AND NITROGEN IN ACROMEGALY.*

By OLAF BERGEIM, Ph.D., F. T. STEWART, M.D., and P. B. HAWK, Ph.D.

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Marie (1) was the first to call attention to the relation of acromegaly to hypophyseal tumor, and he believed the disease to be a manifestation of the decreased function of this gland. This view was supported by various observers, but at the present time it has been largely given up and replaced by the theory of a hypersecretion of the anterior lobe as a necessary antecedent of acromegaly. Among the facts supporting this may be mentioned the following. All tumors of the hypophysis do not result in acromegaly, but only the adenomas. Destructive tumors do not evoke symptoms of the disease (Fischer (2) and others). This is supported by surgical experience. Hochenegg (3) first showed that removal of the hypophyseal tumor in a typical case of the disease could lead to a rapid recovery.

The apparently beneficial effects of pituitary extract administration have been due to alleviation of subjective symptoms and not to alterations in the fundamental growth disturbance (Biedl (4)). The conflict of views has been considerably clarified by the suggestion of Tamburini (5) that two pathological processes were involved, one leading to an hypertrophy of the gland and to acromegalic symptoms, and being followed by atrophic changes with cachectic symptoms. The widely varying results and differing conclusions as to the metabolism in acromegaly and the effects in general of the hypophysis upon nutrition are probably due to the failure to recognize the occurrence of different stages in the disease.

A retention of calcium and phosphorus has been found in acromegaly by Rubinraut (6), Edsall and Miller (7) in the progressive stage, Parhon (8), Medigreceanu and Kristeller (9), and Moraczewski (10). Tauszk and Vas (11) found an increased excretion of calcium but a slight retention of nitrogen and phosphorus. Franchini (12) obtained a retention of nitrogen, calcium, and magnesium, with a loss of phosphorus and chlorine. Schiff (13) found a retention of phosphorus in a case of acromegaly with myxedema. Oberndorfer (14) believes that there are no marked changes in metabolism. In his case there was a slight loss of calcium and a gain of phosphorus. Most of these authors found slight retentions of nitrogen and of chlorine. Medigreceanu and Kristeller noted a slight retention of sulphur, but Rubinraut found a loss

* Received for publication, June 12, 1914.

of this element. It is apparent that the agreement of results is not so marked as to make further work unnecessary. If it be true, as seems probable, that there is a primary progressive stage of acromegaly and that this terminates in a resting or retrograde stage, some of these variations may be accounted for.

Work has been done to determine the effects of hypophysis treatment in this disease. Schiff found an increase in phosphorus excretion in acromegaly after treatment with pituitary tablets; in normal persons this was not noted. In a case complicated with glycosuria Moraczewski noted after similar treatment a negative nitrogen and chlorine balance with little change in calcium and phosphorus excretion. Rubinraut found that hypophysis feeding increased the excretion of calcium and phosphorus. Tauszk and Vas noted no particular effect. Medigreceanu and Kristeller in two successive periods of feeding of the anterior lobe noted distinct losses of phosphorus and of calcium, and a loss of nitrogen.

Similar experiments have been made on lower animals. Thompson and Johnston (15) fed dried glands to dogs and noted an increased excretion of nitrogen and phosphorus in the urine. Falta, Bolaffio, and Tedesko (16) found pituitrin to have the same effect as thyroid extract. It increased the elimination of calcium and magnesium through the feces. Oswald (17) observed no change in the nitrogen and phosphorus excretion of dogs. Malcolm (18), also working on dogs, found that feeding the posterior lobe caused a phosphorus loss, while the anterior lobe caused a nitrogen and phosphorus gain with a loss of calcium and magnesium. The administration of fresh gland had apparently nearly the opposite effect.

Working with rabbits Franchini (19) noted an increased excretion of calcium, magnesium, and phosphorus. Mochi (20) likewise by subcutaneous injections obtained increased elimination of calcium and phosphorus, which he ascribed to catabolism of bone.

In hypophysectomized animals there seems to be a greatly increased excretion of nitrogen and of phosphorus (Narboute (21) and Wolf and Sachs (22)).

Closely related to these findings are the effects on growth noted during hypophysis treatment. Cerletti (23) found that injection of extracts caused the body-weight to remain almost stationary. Sandri (24) also found a slowing in growth of mice fed on hypophyseal substance. Aldrich (25) noted no marked changes in young dogs. Schäfer (26) found that dried extract of the anterior lobe caused young rats to grow more rapidly.

The diversity of results obtained with gland extracts is not difficult to understand when we realize the different conditions under which they were obtained. The extracts were made in different ways and in most instances from mixtures of posterior and anterior lobes which possess distinct influences on metabolism. The results obtained with anterior lobe extracts should be of more value as this is the portion which normally influences growth, while the posterior portion may seriously disturb metabolism. The results in acromeg-

aly must also vary with the stages of the disease and other conditions.

The bone changes in acromegaly, according to Biedl, are not so marked as clinical appearances would suggest. There is no special disease process involved, but a deposition and resorption of bone substance takes place just as in normal bone growth.

The present study was made in the wards of the Jefferson Hospital on a man who was suffering from a typical early and progressive form of acromegaly.

The points of interest in the case reported may be briefly summarized as follows:

J. S. Male, aged 35 years, native of Russia; barber. Admitted to the Jefferson Hospital, Nov. 17, 1913, and discharged without change in condition, Dec. 9, 1913. He had never had any serious illness but had suffered during the past fifteen years from a catarrhal condition of the nose.

Physical Examination.—Well developed, well nourished, with typical acromegalic aspect. Head large and well developed. Eyes showed contracted pupils and reacted slowly to light and to accommodation. Nose large and broad at tip. Mouth and ears normal. No separation of upper teeth but slight separation of lower teeth especially about right lower canines. The arteries were hard and tortuous. The hands were very large, the fingers large and thick, and the ends blunt and sausage-like. Skin very thick. Sensation impaired in fingers, especially on dorsal surfaces.

Röntgen ray showed true acromegaly. The sella turcica was 7/8 of an inch long, and 7/8 of an inch deep. The posterior clinoid processes were somewhat atrophied.

Eye examination showed vision $o'' = 20/30$. The pupils reacted to light convergence. The tensions were normal, and ocular movements were unimpaired. The ophthalmoscopic examination was negative. Examination of nose and throat showed deflected septum and large submerged tonsils which were operative.

METHOD OF STUDY.

The details of food preparation and collection were carried out with the careful coöperation of the patient and of the hospital staff. The patient was placed on a uniform diet, amounts of each food given being based on the patient's choice during a preliminary day. The diet consisted of milk, graham and soda crackers, cereal, salt, sugar, water, butter, meat, tea, and coffee, and during the three final days also five grams of agar-agar per day. The meat was prepared previous to the experiment, thoroughly mixed, boiled, and

again thoroughly mixed, placed in glass jars, sterilized, and kept in a frozen condition until used. Aliquot portions of the foods were taken for analyses.

Urine was collected in exact twenty-four-hour samples and kept in an ice box. Thymol was used as a preservative. The feces were separated by the use of carmin and kept in thymolized friction top cans and in a frozen condition until the end of the period when they were thoroughly mixed for analysis.

Calcium and magnesium were determined by McCrudden's methods (27). Sulphur in the urine was determined by Benedict's method (28). Sulphur in the feces and foods was determined after oxidation with fuming nitric and hydrochloric acids and with Benedict's sulphur reagent. Phosphorus was determined by Neumann's method. Nitrogen was determined by the Kjeldahl method.

DISCUSSION.

The most striking fact brought out by the balance of the five elements in our case of acromegaly is the retention of calcium oxide, magnesium oxide, and phosphoric anhydride, of 16.2, 20.6, and 15.3 per cent. respectively (table I). The absolute retentions of

TABLE I.

Balance of Calcium, Magnesium, Phosphorus, Sulphur, and Nitrogen in Acromegaly.

	Calcium oxide.	Magnesium oxide.	Phosphoric anhydride.	Sulphur.	Nitrogen
	Grams.				
Ingestion daily	1.494	0.486	3.192	1.190	18.84
Excretion urine	0.159	0.160	1.701	1.006	17.60
Excretion feces	1.093	0.226	1.002	0.135	1.10
Excretion total	1.252	0.386	2.703	1.141	18.70
Retention daily	0.242	0.100	0.489	0.049	0.14
Retention per cent	16.2	20.6	15.3	4.1	0.7

these elements, 0.242 of a gram of calcium oxide, 0.100 of a gram of magnesium oxide, and 0.489 of a gram of phosphoric anhydride, cannot be considered high, but are nevertheless distinct. The fact that the sulphur and nitrogen metabolism shows a practical equilibrium emphasizes these retentions, as does also the fact that there is

a rough parallelism in the retentions of these bone-forming elements. There would seem, therefore, to be a disturbance in bone growth not secondary to changes in general metabolism, probably leading to increased formation of osseous substance.

Whether the bone thus formed be normal bone, as has been suggested, or abnormal bone, as believed by Edsall and Miller, cannot be decided from the data at hand. The fact that magnesium oxide and phosphorus were retained in amounts more than sufficient to form bone with the retained calcium oxide is of interest, as a similar condition was found by Edsall and Miller and others for the element phosphorus. Probably, however, too much emphasis cannot be laid upon these variations, and the most direct procedure here would be the analysis of bones from this condition.

TABLE II.

Daily Excretion of Calcium, Magnesium, Phosphorus, Sulphur, and Nitrogen in Acromegaly.

Urine.

	Day.							Daily average.
	1	2	3	4	5	6	7	
	Grams.							
Calcium oxide	0.064	0.131	0.163	0.190	0.085	0.225	0.256	0.159
Magnesium oxide	0.032	0.220	0.144	0.250	0.043	0.191	0.242	0.160
Phosphoric anhydride	1.672	1.972	1.572	1.666	1.577	1.715	1.735	1.701
Sulphur	1.004	1.009	1.003	1.010	1.003	1.008	1.003	1.006
Nitrogen	15.38	17.25	18.13	18.26	17.26	17.74	19.15	17.60

Feces.

Daily averages (grams).

Calcium oxide.	Magnesium oxide.	Phosphoric anhydride.	Sulphur.	Nitrogen.
1.093	0.226	1.002	0.135	1.10

The ratios of the distribution of the excretion of the mineral elements between the urine and the feces are for calcium oxide 1 to 6.9, for magnesium oxide 1 to 1.4, and for phosphoric anhydride 1.7 to 1. These are within normal limits and suggest that absorption and excretion of these elements are carried out in about the normal way. The same can probably be said for the elements sulphur and nitrogen, which are as usual eliminated almost entirely by way of the kidneys. That the absolute amounts of calcium oxide and mag-

nesium oxide eliminated by the urine are almost exactly identical is probably a coincidence.

A fact probably without particular relation to acromegaly but of great importance from the standpoint of general calcium and magnesium metabolism is brought out by table II. Pronounced variations in the daily urinary output of these elements will be noted, although the patient was on a strictly uniform diet, and the urine was collected at exact twenty-four-hour intervals. Further, the sulphur and nitrogen excretions are uniform. Nothing could show more clearly the dangers of judging the metabolism of these elements from urinary analyses alone. Take for example the excretion of calcium oxide on days 4, 5, and 6, 0.190, 0.085, and 0.225; and of magnesium oxide, 0.250, 0.043, and 0.191 on the same days. The explanation of these variations is not entirely clear, but the possibility has suggested itself as worthy of consideration that there is a relation between the absorption, particularly of calcium, and the accumulation in the intestine of residual material rich in salts soluble with difficulty. This would probably be particularly true on a diet containing considerable milk with its high content of calcium salts. In the study under discussion no feces were passed until the fifth day, and there is a steady rise in urinary calcium excretion until that day when there is a pronounced drop followed by marked rises on the succeeding days. It would seem desirable to consider this point in connection with studies of calcium metabolism, as, if supported further, it would tend to show reports of increased or decreased urinary output of these elements to be of very doubtful value. It may be, however, that these variations are particularly notable in acromegaly due to an increased absorptive power of the intestine for calcium salts in this condition.

The authors wish to express their obligation to Miss Gilson, Miss Looman, and Miss Troester for their aid during the progress of the investigation.

SUMMARY.

A distinct retention of calcium, magnesium, and phosphorus was noted, which was not accompanied by corresponding changes in general metabolism, as evidenced by a practical balance of nitrogen and sulphur.

It is concluded that there is a primary disturbance in the metabolism of the mineral elements mentioned, with the probable formation of new bony tissue.

The distribution of elements between urine and feces shows no abnormal variations.

A suggestion is made as to the reasons for variable calcium output in the urine on a uniform diet.

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CALCIUM METABOLISM AFTER THYRO-PARATHYROIDECTOMY.*

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That the thyroid apparatus, particularly the parathyroids, either directly or indirectly exerts considerable influence over the metabolism of calcium has been clearly shown in a number of ways by several authors. Probably the most striking of these experiments are those made by Erdheim (1) on rats. He observed that after parathyroidectomy the dentine of the rapidly growing incisor teeth ceased to calcify. After successful transplantation of the glands the calcification process again commenced and a zone of uncalcified dentine marked the period during which the parathyroids were absent. Erdheim and Canal (2, 3) also found that callus formation was greatly retarded, due almost entirely to deficient deposition of calcium salts. He also cites the relative frequency of parathyroid hyperplasia in osteomalacia, as well as changes in the teeth in this disease, similar to those noted by him in the animals mentioned.

Biedl (4) found that thyroidectomy prevented the normal development of the bony skeleton in animals, and Bircher (5) showed that treatment with thyroidin in these cases led to a more rapid formation of bone and a hastened process of calcification. Towles (6) found no special peculiarities in the calcium metabolism of exophthalmic goitre. Analyses of the blood and of various tissues have been made after parathyroid removal but these show a lack of uniformity. MacCallum and Voegtlin found a decrease of calcium in the blood and brain of dogs killed in parathyroid tetany, but not after incomplete removal (7). This has been confirmed by Pexa (8) and Aschenheim (9). Analyses by Cooke (10), however, indicate an increased calcium content of the brain under these conditions. This author found little change in the calcium metabolism. Leopold and von Reuss (11), in their studies of the calcium content of the tissues of normal and parathyroidectomized rats, found little change with adults and some decrease in young animals. The soft tissues were poorer in calcium and the bones were richer in this element. Similarly Morel (12) found that parathyroid extracts favored the growth of bone in young, but not in old animals. Neurath (13) found an increase in the calcium precipitable by oxalate in the blood in animals after parathyroidectomy. Most authors claim that an increased excretion of calcium in the urine follows this operation. Among these are MacCallum and Voegtlin (14) and Frouin (15). Musser and Goodman (16), however, found a marked decrease in the urinary calcium, as did also von Reuss and Welde. Cooke likewise found no increased excretion of calcium in the urine.

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The relation of calcium metabolism and the parathyroids has been studied most extensively in connection with the tetany brought on by their removal. The finding by MacCallum and Voegtlin that injection of calcium salts caused a temporary disappearance of the symptoms has been confirmed by a number of investigators. Some doubt is expressed as to any actual prolongation of life by this treatment. Arthus and Schaffermann (17) apparently obtained somewhat more persistent results in rabbits with calcium chloride ingestion. That this power is not restricted to calcium salts has been shown by Berkeley and Beebe (18) and by Canestro (19), who obtained similar results with magnesium and strontium salts, by Frouin (20) with lanthanum and thorium salts, and by Joseph and Meltzer (21) for sodium chloride solution. Further, simple bleeding decreases the symptoms. On the other hand, it has been shown that the injection of calcium precipitants causes a more rapid oncome of tetany (22).

Numerous attempts have been made to correlate the above findings with the tetanies of man presumably of parathyroid origin. In infantile tetany von Cybulski (23) found a much less calcium retention during the period of tetany. Schwarz and Bass (24) obtained similar results. Schabad (25) in a case of rachitis with tetany found no changes from the metabolism of rachitis alone, nor were the symptoms alleviated by treatment with calcium salts. Phosphorized oil, however, caused a retention of calcium and an improvement of the tetany. Haskins and Gerstenberger (26) could not show that in man parathyroid and calcium salts favored calcium retention. The theory of Stoeltzner (27) that tetany is due to a heaping up of calcium in the organism has met with much objection. On the other hand, Cattaneo (28) found a decreased calcium content of the blood in six cases of spasmophilia, and it has been suggested that the tetanies of pregnancy may be due to the drain on calcium that occurs at this period. Parathyroid tetany also develops very quickly in lactating animals (Carlson (29)).

The therapeutic application of calcium treatment of tetany in human beings has met with some success (30, 31, 32). The relation of infantile tetany to calcium metabolism is further supported by the work of Quest (33) who found a low calcium content of the brains of children who died in tetany. This was confirmed by Silvestri (34) but not by Cohn (35) or by Leopold and von Reuss.

With regard to the inevitability of the oncome of tetanic symptoms in various animals and in man after complete removal of the thyroid apparatus there has been much dispute, although the opinion more generally held seems to be that the results are always fatal and that any other termination is to be ascribed to the incompleteness of the operation, or insufficient length of observation. Much evidence has, however, accumulated in support of the view, emphasized particularly by Vincent (36), that a considerable percentage of animals of different species may survive total extirpation for prolonged periods and may show no specific symptoms of any kind; and also that the operation produces very different results in different species, in general the carnivora being much more sensitive than herbivorous animals. Vincent concluded that in monkeys neither thyroids nor parathyroids were essential to life. Breisacher (37) found that dogs fed on a milk diet suffered less than those on a meat diet. Vassale and Generali (38) found further that the tetany induced by thyroidectomy is

less marked in old than in young animals. In hunger the tetany was much less marked also than on a meat diet.

Marine (39) apparently goes so far in the belief that the parathyroids are essential as to make the absence of symptoms after removal a test for the presence of accessory glands. Parathyroidectomy extending over a long period was less often fatal and calcium salts sometimes tided an animal over an otherwise fatal tetany. Why this should be taken as a sign of compensation by accessory tissue rather than by some other gland or set of glands does not seem clear. Paton (40) has suggested that "the variations in symptoms may be due to the fact that removal of parathyroid reduces the stability of the central nervous system but that some other disturbing factor, for example, pregnancy, is required to upset it sufficiently to cause the manifestation of the symptoms."

The observations of Gozzi (41) and others (42, 43), that thyroparathyroidectomy was less rapidly fatal than removal of parathyroids alone, do not agree with the findings of Halpenny (44), Wiener (45), and Biedl (46). Other work done along this line will be mentioned in the discussion of our results.

In connection with the calcium metabolism the findings of Greenwald (47) should be mentioned. This author found an increase in the total phosphorus of the blood and serum after parathyroidectomy, the phosphorus being mainly in an ether-insoluble, acid-soluble form. He found likewise (48) a decrease of the phosphorus of the urine after operation with an increase as tetany came on. A pronounced increase in the creatinin content of the urine was noted. Frontali later observed (49) a decreased urinary creatin output after thyroidectomy. The latter also found a decrease of creatinin in the muscles and an increase in the blood.

Considerable study has been made of the nitrogenous metabolism after parathyroidectomy, particularly with the idea in view of proving or disproving an intoxication as the basis of the symptoms.

MacCallum and Voegtlin found a great increase in the ammonia content of the blood. Similar results were obtained by Cooke, Morel (50), and Medwedew (51), but could not be confirmed by Carlson and Jacobson (52), Albertoni (53), Greenwald, Musser and Goodman, and von Reuss and Welde. Increases of the ammonia content of the urine have been found by Berkeley and Beebe. Frouin (54), Cooke, and Morel. Greenwald found only a slight increase of ammonia but a larger increase of nitrogen of unknown character. Other abnormalities noted by Cooke were increases in the excretion of magnesium, and the presence of lactic acid due probably to the muscular work of tetany.

After thyroidectomy alone there is usually a reduction of organic as well as inorganic metabolism. However, Falta, Bolaffio, and Tedesko (55) found a pronounced increase in the nitrogen: phosphoric anhydride ratio, due to increased nitrogen excretion and decreased phosphorus excretion in the urine. Thyroidin treatment brought about an increased excretion of calcium in the feces. Similarly Scholz (56) found in man that such treatment brought about an increased phosphorus excretion in the feces. Kottmann (57) found a lowering of the freezing point of the blood in Basedow's disease, due supposedly to abnormal content of metabolic products.

The present study was carried out on a patient in the Jefferson Hospital.

Clinical History.—G. H., aged 32 years, colored; laborer. Admitted to the surgical ward of the Jefferson Hospital, Oct. 19, 1913. Fourteen months before this he began to experience difficulty in breathing, which increased steadily for two months, when a low tracheotomy was performed by a surgeon in Florida, where the patient was then residing. The condition evidently was thought to be syphilis of the larynx, as the patient was subsequently given salvarsan, and later neosalvarsan, potassium iodide, and inunctions of mercury. After coming to Philadelphia the patient entered Dr. D. Braden Kyle's ward at the Jefferson Hospital. Dr. Kyle found a growth in the larynx and removed a portion of this growth for microscopic examination. Histologic study of the excised fragment revealed a typical picture of papilloma. From a clinical standpoint, however, Dr. Kyle regarded the growth as malignant, and because of its extent transferred the patient to the surgical ward. There was nothing relevant in the family history. The patient had had gonorrhea about five years before his laryngeal trouble, but had never had a chancre or other evidence of syphilis. The Wassermann reaction was negative. There had never been much pain, never any hematemesis. The tracheotomy tube was worn constantly, and when this was closed with a finger the patient was able to whisper. The man was well nourished; the temperature, pulse, and respiration were normal. The pupils reacted to light and accommodation. There was a large cauliflower-like mass filling the glottis and extending up into the epiglottis and into the esophagus. On palpation the larynx was found to be considerably expanded, measuring three inches at the widest part. It could be moved laterally, and it moved when the patient swallowed. The thyroid gland was enlarged and hardened. Examination of the thorax, abdomen, extremities, and urine showed nothing abnormal.

An operation was performed on Oct. 21, 1913, intratracheal insufflation of ether being employed to maintain anesthesia. A median incision was made from above the hyoid bone to the tracheotomy opening, and a transverse cut made at each end of this incision, thus forming lateral flaps, which were turned outward. After separating the larynx from the soft parts on each side, it was discovered that the growth had invaded the thyroid gland, the trachea, and the esophagus. The sternohyoid and sternothyroid muscles were severed near the sternum, the omohyoid beneath the sternomastoid, and the trachea in the episternal notch below the tracheotomy opening. A tube was then passed into the esophagus through the mouth, and the anterior half of the esophagus, from the sternum to the pharynx, separated by sharp dissection while the specimen was dragged upwards to facilitate the incisions in the esophagus, which incisions were continued up into the pharynx to meet in the median line above the hyoid bone. The excised mass of tissue included a portion of the pharynx, the anterior half of the esophagus as low as the sternum, the hyoid bone, the epiglottis, the larynx, the trachea as far as the sternum, the entire thyroid gland, and of necessity the parathyroid glands, although it must be stated that owing to the neoplastic infiltration neither Dr.

Coplin nor myself could identify the parathyroids in the excised tissue. After the growth had been removed, the esophagus and the pharynx were sutured over the tube with catgut, and the skin flaps brought together except at the points corresponding to the outer angles of the lower transverse incision, where gauze drains were inserted. The tracheal stump was anchored to the skin with heavy silk thread. The operation lasted one and one half hours, and was followed by little shock. The patient sat up the next day and was out of bed at the end of the first week. Liquid food was given through a tube which reached to a point just below the lowest suture in the esophagus. On the eighth day the lower part of the esophageal wound began to leak and some of the food passed into the trachea. Subsequent to this nourishment was administered through a long tube which passed through the nose, pharynx, and esophagus into the stomach. The tube was not removed between feedings, because of the difficulty of guiding it past the opening in the lower cervical esophagus. During the first postoperative week the patient coughed only occasionally, but subsequent to the entrance of food into the trachea the cough became more frequent, the expectoration being tough stringy mucus. On Nov. 4, 1913, two weeks after the operation, sharp pain due to pleuritis was felt in the right chest. The respirations increased in frequency and fever developed. Later signs of fluid in the pleural cavity became evident, but none could be obtained by aspiration. On Nov. 21, 1913, an incision was made into the pleural cavity and a large quantity of foul pus evacuated. One week after this and thirty-eight days after the laryngectomy the patient died.

Autopsy.—A septic pneumonia of the right lung and gangrene of the right pleura were revealed. The laryngeal growth proved on microscopic examination to be a squamous-celled epithelioma.

METHOD OF STUDY.

The patient was kept in a private room in the Jefferson Hospital and under charge of a private nurse throughout the period of study.

On account of the necessity of feeding the patient through a nasal tube the food given was all in liquid form. It consisted of soup, eggs, milk, orange juice, and water. No attempt was made to have the amount of each food uniform from day to day but the amounts fed were accurately measured and aliquot portions were taken at each meal for analysis. The food samples for the day were mixed in the proportions as fed, evaporated to dryness, ground to a fine powder in a mortar, and thoroughly mixed. Portions of these powders were taken for analysis. Water was analyzed separately. The urine was carefully collected in twenty-four-hour periods, and calcium determinations were made in each day's output. Feces separation was made by means of carmin and was not attended with

difficulty. Feces were kept in a friction top can in a frozen condition until the end of the period when they were thoroughly mixed and portions taken for analysis. McCrudden's (58) method for the determination of calcium was employed.

Blood samples of fifty cubic centimeters each were obtained on the first and final days of the test period. Coagulation was prevented by means of potassium oxalate. The calcium was determined gravimetrically with platinum crucibles, by a modification of McCrudden's method for pure solutions.

The results obtained are given in table I.

TABLE I.

Ten-Day Calcium Balance after Thyroparathyroidectomy.

Day.	Calcium oxide in food.	Calcium oxide in urine.		Calcium oxide in feces.	Total excretion.
		Volume.	Calcium oxide.		
1	1.0923 gm.	635 c.c.	0.0051 gm.		
2	0.7610 gm.	870 c.c.	0.0074 gm.		
3	1.6355 gm.	865 c.c.	0.0091 gm.		
4	1.5258 gm.	900 c.c.	0.0131 gm.		
5	0.7036 gm.	740 c.c.	0.0078 gm.		
6	1.6912 gm.	1,160 c.c.	0.0145 gm.		
7	2.3261 gm.	940 c.c.	0.0193 gm.		
8	2.2882 gm.	1,125 c.c.	0.0169 gm.		
9	1.9273 gm.	1,110 c.c.	0.0244 gm.		
10	2.7847 gm.	1,765 c.c.	0.0159 gm.		
Period	16.7357 gm.		0.1335 gm.	16.1444 gm.	16.2779 gm.
Daily average...	1.6736 gm.		0.0134 gm.	1.6144 gm.	1.6278 gm.

Balance.

10 days ..... 0.4578 gm. of calcium oxide retained.

Balance.

Daily average ..... 0.0458 gm. of calcium oxide retained.

Retention ..... 2.74 per cent. of calcium oxide.

Blood.

First sample... 0.0087 per cent. of calcium oxide.

Second sample. 0.0100 per cent. of calcium oxide.

DISCUSSION.

It will be noted that there was a retention during the ten-day period of 0.4578 of a gram of calcium oxide, or 0.0458 of a gram per day. This corresponds to a percentage retention of 2.74 per cent. of an ingestion of 1.6736 grams of calcium oxide per day. A retention as slight as this on a comparatively high intake cannot be said to show after operation a distinct tendency of the body to take up

calcium salts. In fact it is probable that a normal individual on such a diet would tend to retain more rather than less calcium oxide than was noted in this case. The figures must therefore be taken to show an approximate calcium equilibrium. They have a certain negative significance in that they certainly give no support to any assumption of a pronounced calcium loss to the body in this condition.

The relation of the excretion of calcium in the urine to that in the feces was found to be approximately 1 to 121. This proportion is lower than was found by us in osteitis deformans or by McCrudden and Fales (59) in intestinal infantilism. It is in fact one of the most striking facts brought out by this table. Taken in connection with the low figures for calcium in the blood the most plausible interpretation is that absorption of calcium by the intestine must have been very slight. That there was a demand by the body for calcium salts to fight off infection or toxemia is indicated by the fact that three times as much calcium was retained as was excreted in the urine, small as this amount was. There must have been deficient absorption which must be attributed to the thyroid and parathyroid removal. How such an effect could be produced is problematical. In the apparently somewhat related intestinal infantilism McCrudden and Fales could not find that the poor absorption was secondary to the overproduction of phosphate, fatty or volatile acids, nor could they determine the form in which about one half of the calcium of the feces was combined. Of course the diet employed in our case, consisting largely of eggs and milk, contained considerable organic phosphorus and the possibility of a faulty digestion of these substances on calcium absorption must be considered, particularly in view of Dibbelt's experiments (60). He found that the introduction of sodium bicarbonate or disodium phosphate directly into the intestine of animals caused an increased excretion of calcium in the urine. He assumed that excessive amounts of these calcium-precipitating agents might be formed by decomposition of undigested casein and from carbohydrate fermentation and believes that the etiology of rachitis must be of this nature. We are not, however, aware of any evidence proving that this actually occurs. The fact that Keeton (61) has shown in

cats that parathyroidectomy greatly diminishes gastric secretion is of interest in this connection. A lack of hydrochloric acid secretion might operate in two ways to decrease calcium absorption. First, it decreases the amount of calcium chloride or acid phosphate formed from the carbonate or phosphate of the diet. Secondly, intestinal fermentation and putrefaction processes of a nature calculated to form calcium precipitants would be increased due to the decreased antiseptic power of the gastric juice. It would be of interest to know whether an achylia exists in intestinal infantilism, rickets, and allied disorders. It is our intention to investigate this point.

It must be borne in mind, however, that we know little with regard to the mechanism of calcium absorption. If we assumed that calcium was absorbed largely as chloride or acid phosphate in the stomach or upper intestine, the problem would be simplified. We have, however, noted in a study on acromegaly (61) that urinary excretion of calcium increases when calcium-rich feces accumulate in the intestine, indicating absorption of this element from the large intestine. It appears that in the present state of our knowledge we cannot bar out a more direct parathyroid action, through the intermediary of a nuclein metabolism or on the intestines themselves.

The slight increase in urinary calcium during the period may be considered as largely due to a higher calcium ingestion, possibly also to a slight constipation. The somewhat higher blood calcium content at the close of the period may be attributed to similar causes. Possibly also there has been a greater demand on calcium for antitoxic purposes and deficient excretion of calcium phosphate.

Why in the case of complete thyroid and parathyroid removal under consideration did not tetanic symptoms develop during the period of thirty-nine days after operation? Some authors who have worked particularly upon carnivorous animals, such as the dog, would unhesitatingly answer that accessory parathyroids must have been present. From the nature of such an assertion, precluding, as it does, either proof or disproof, and assuming as it does the point in question, it follows that this argument must be given not first but last consideration, and resorted to only if other explana-

tions are not forthcoming. Unfortunately the explanation of the absence of tetany in the present case cannot be given with any certainty. Possible explanations only can be suggested.

Our patient was on a high calcium diet. Absorption was not satisfactory, but there was a retention and not a loss of this element. Certainly these facts are significant. Calcium salts delay the oncome of tetany. Most observers have, however, found this inhibiting effect of calcium salts to be temporary. It could hardly be expected to extend over a period of more than a month.

The patient was further kept quiet and thus untoward influences were avoided. The suggestion of Paton that parathyroid removal reduces the stability of the central nervous system, but that other disturbing factors may be required to upset it sufficiently to cause symptoms, has already been mentioned. The facts that carnivora are more sensitive than herbivora, that dogs fed on a milk diet suffered less than on a meat diet, and that in old animals the tetany was less marked than in young animals, have already been considered.

Bircher (62) has reported complete and permanent recoveries from tetany following partial removal of thyroids and parathyroids, by means of temporary treatment with parathyroid extracts. Schneider (63) reports a case of neck tumor of the thyroid in a woman of 46 years, in which apparently complete thyroid and parathyroid removal was followed by acute tetany which was overcome by treatment with parathyroid extracts. This case as well as ours may of course be explained on the basis of the supposed presence of supernumerary parathyroids. It would seem more plausible that this compensation was brought about by hypertrophy of some other of the ductless glands, probably the hypophysis. This view is supported by the known relation of this gland to calcium metabolism, its rapid hypertrophy in the calcium-mobilizing period of pregnancy, and after thyroid removal, as shown by Rogowitsch (64) and others (65); also after parathyroid removal as reported by Thompson (66), Pepere (67), and Halpenny (68). It may be more than an interesting coincidence that hypophysectomy (Crowe, Cushing, and Homans (69)) is much more rapidly fatal in old than in young animals, while parathyroidectomy (Vassale and Generali (70) and others) has a higher mortality in the young when apparently the compensatory mechanism is not so well developed. Ott and Scott (71) have further found that pituitary extracts temporarily inhibit parathyroid tetany. The observations of Thompson, Leighton, and Swarts (72) are of interest in this connection. They found that ligatures producing slow interference with the blood supply led to no tetanic symptoms, but that slow wasting occurred.

The fact that the parathyroids in our case were so infiltrated as

to be unrecognizable is very important as suggesting that there had probably been a gradual decrease in parathyroid activity with sufficient lapse of time for a compensatory mechanism to develop sufficient to prevent the oncome of tetany at least for a considerable period of time, but not sufficient to preserve a normal metabolism of calcium.

The authors wish to express their indebtedness to Dr. Kinnaird, Miss Looman, and Miss Rowe for their coöperation.

CONCLUSIONS.

The metabolism of calcium was studied in a man after complete removal of the thyroid and parathyroid glands. A slight retention of calcium (0.4578 of a gram of calcium oxide in the ten-day period) was noted. The urinary calcium excretion was low, averaging 0.0134 of a gram per day on a daily ingestion averaging 1.6736 grams of calcium oxide. A slight increase was observed during the period of study in the calcium content of the blood.

No symptoms of tetany were noted in the patient, who survived operation thirty-nine days.

The low urinary and blood calcium values are taken to show deficient absorption of calcium, which may bear some relation to the decreased gastric secretion after parathyroidectomy.

Attempts are made to explain the non-occurrence of tetany as due to the high calcium intake and to the development of a compensatory mechanism in which the pituitary body may play a part.

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A METHOD FOR ESTIMATING THE BACTERIA IN THE CIRCULATING BLOOD IN RABBITS.*

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Not a few of the determinations in experimental bacteriology have to do with the testing of bacteria for their infectious power. The laboratory animals principally employed for this purpose are small, and afford few reliable criteria which can be used to decide the result. The criterion which, because of its very definite character, is usually relied upon is the development of severe illness and the intervention of death; but this criterion cannot take account of degrees of intensity of infection, except as they affect the period of survival of the animal after inoculation, which is manifestly a coarse differential. All who have conducted so called virulence tests with bacteria have felt the need of other means of differentiation than mere survival or death of the inoculated animal. In the course of some experiments on the therapeutics of streptococcal and pneumococcal infections, this need became imperative, so that a means was sought that should yield data capable of comparison at different periods and intervals after inoculation, and which did not rely alone on the ultimate result of recovery or death.

The starting point of the quest was the well known phenomenon, first observed by von Fodor[1] and investigated accurately by Wyssokowitsch,[2] namely, that bacteria injected into the circulation are soon filtered out of the blood and, according to their degree of infectiousness, are destroyed or reappear, multiply, and, when of sufficient power, ultimately cause death. Hence the question which arose was whether the disappearance, reappearance, and multiplication proceed with a degree of regularity and consistency rendering a numerical estimate feasible of the bacteria in the circulating blood.

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[1] von Fodor, J., *Arch. f. Hyg.*, 1886, iv, 130.

[2] Wyssokowitsch, W., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1886. i. 3.

The later studies of Weil[3] and of Reichstein,[4] which deal with the fate and the estimation of streptococci within the blood stream, offered an encouraging affirmative on this point, so that the next question to arise was whether blood taken repeatedly from superficial vessels sufficed for the purposes of the estimation.

EXPERIMENTAL PART.

Bacteria and Injections.—The streptococcus used in the following experiments was originally isolated from the throat of a scarlet fever patient. It is a typical hemolytic, Gram-positive, chain-forming streptococcus. It has been repeatedly passed through rabbits and, at the beginning of this work, 0.25 of a cubic centimeter of a twenty-four-hour bouillon culture[5] per kilo of body-weight killed rabbits within one to five days. The pneumococcus used belongs to group I according to Neufeld's classification. Its virulence has been maintained by repeated passages through mice and rabbits. 0.001 of a cubic centimeter of a twenty-four-hour bouillon culture per kilo of body-weight kills rabbits within one to three days. In all experiments the bacteria were injected into the ear veins.

Taking the Blood.—The uninjected ear was carefully shaved and washed with 95 per cent. alcohol. The marginal vein was pricked and a few drops of blood allowed to flow out before any was taken for the cultures. Then a definite number of drops were caught directly into sterile Petri dishes. A tube of agar, previously melted and cooled to 42° C., was immediately poured into the dishes and the blood and agar were thoroughly mixed. Two or more plates

[3] Weil, E., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1911, lxviii, 346.

[4] Reichstein, S., *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1913, lxxiii, 209.

[5] We have found blood bouillon (3 to 5 drops of sterile defibrinated rabbit blood in a tube of 5 c.c. of beef infusion bouillon) to be an excellent medium for streptococci and pneumococci. The difficulty of "infecting" large quantities of medium with loop inoculations from bouillon to bouillon is eliminated (Gillespie). The bacteria do not lose this virulence as readily in this medium as in ordinary bouillon. Another advantage is that the bacteria live in this medium for long periods of time without being transferred. Streptococci will live for 6 months when the cultures are kept in the dark at low temperature; it is not necessary to seal the tubes in any way. Pneumococci will live for at least 6 weeks. This medium was used throughout the experiments instead of plain bouillon.

were made from each rabbit, the amount of blood varying from one to ten drops, according to the supposed degree of infection. In this way plates were obtained on which the colonies could be easily counted and the various plates compared.

The above procedure permits of frequent taking of blood with the least possible disturbance to the rabbit. Contaminations rarely occur if the ear is properly cleansed and a few drops are allowed to flow out before taking the blood for the cultures. Blood from the first few drops adheres to the surface of the ear and the following drops run over this without coming in contact with the skin. The accuracy of the method might be objected to, because the measuring of the blood is as inaccurate as the size of one drop of blood may differ from another. If the veins are pricked by a stab with the same sharp-pointed instrument and the surface of the ear is held in a perpendicular position so as not to allow the blood to collect in large quantities before flowing off, drops of fairly constant size can be obtained and the number of colonies on various plates of the same number of drops is remarkably uniform. The accuracy of the procedure can also be tested by infecting a series of rabbits with the same quantity of a bacterial suspension per kilo of body-weight and taking cultures at stated intervals in a short time after the injections. Experiment 1, as given below, was done with this object in view.

STREPTOCOCCUS INFECTIONS.

Experiment 1.—Each of seven rabbits was injected intravenously with 0.25 c.c. of a fresh bouillon culture of streptococci per kilo of body-weight. Cultures were made from the opposite ear 30 minutes and 5½ hours afterwards. In this, as in all other experiments, the number of colonies was calculated on the basis of ten drops of blood. The results are given in table I.

TABLE I.

	Rabbit 1.	Rabbit 2.	Rabbit 3.	Rabbit 4.	Rabbit 5.	Rabbit 6.	Rabbit 7.
Colonies at 30 min.....	140	145	135	100	130	130	100
Colonies at 5½ hrs.....	8	9	20	22	10	12	10

Many experiments of this character were performed and similar results were always obtained. The first cultures were made after

ample time had elapsed for a thorough and uniform distribution of the bacteria in the blood stream and the number of colonies obtained from the different rabbits shows only slight variations. At the next bleeding, five hours later, the plates were still uniform, although a great reduction in the number of colonies had occurred. These results are probably due to the fact that this initial disappearance of the bacteria from the blood depends largely upon mechanical forces which are about the same in each animal. A biological reaction between the host and the infecting organism had evidently played a very slight rôle up to this time; otherwise, such uniform results could not be expected. Such an experiment also shows that this method of taking blood gives dependable data.

In following the progress of the infections further, it was found that they may take one of three courses, mainly depending upon the virulence of the bacteria. A series of experiments was carried out just after the bacteria had been passed through a number of rabbits. The infections ran an acute course, the blood was never free of bacteria, and the autopsies showed no evidence of localization. After two months' cultivation on artificial media, no animal passages being made, the same amount of bacteria caused a chronic infection in a majority of the animals. There were longer or shorter intervals during which the septicemias were slight or entirely absent. After a time the bacteria suddenly reappeared in the blood and the animals died within a few hours to two days. Autopsies showed localized infections, from which the second blood invasion undoubtedly came. When the bacteria were still less virulent, they permanently disappeared from the blood and the animals recovered. Examples of these types of infection are reported in the following tables.

Experiment 2.—Each rabbit was given 0.25 c.c. per kilo of body-weight of the ame suspension of streptococci into the ear vein. The blood for the cultures was taken from the opposite ear at the times indicated in the protocols. The number of colonies was estimated on the basis of ten drops of blood.

An examination of table II shows that the infections, with the exception of rabbits 2 and 3, ran a remarkably uniform course. From the time of the injections to about five hours afterwards there is a rapid decrease in the number of bacteria. From five to six

TABLE II.[6]

Animal.	Time of bleeding and number of colonies.												
Rabbit 1, weight 1,800 gm.	30 min.	2 hrs.	6 hrs.	10 hrs.	22 hrs.	48 hrs.	72 hrs.	Died 1 hr. after the last bleeding.					
	52	1	18	12	9	100	×						
Rabbit 2, weight 2,000 gm.	30 min.	2 hrs.	6 hrs.	11 hrs.	23 hrs.	30 hrs.	47 hrs.	56 hrs.	Was dead at 64 hrs.				
	200	5	100	200	1000	×	×	950					
Rabbit 3, weight 1,600 gm.	30 min.	2 hrs.	6 hrs.	10 hrs.	22 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	126 hrs.	138 hrs.	Dead at 144 hrs.	
	110	9	13	35	65	1500	×	100	75	×	100		
Rabbit 4, weight 1,500 gm.	30 min.	6 hrs.	18 hrs.	Dead at 36 hrs.									
	25	100	2000										
Rabbit 5, weight 1,750 gm.	30 min.	2 hrs.	6 hrs.	11 hrs.	23 hrs.	30 hrs.	36 hrs.	Dead at 38 hrs.					
	75	5	70	245	×	×	×						
Rabbit 6, weight 1,100 gm.	30 min.	3 hrs.	18 hrs.	24 hrs.	42 hrs.	48 hrs.	Dead at 50 hrs.						
	50	6	1000	2000	×	×							

[6] In the tables, × indicates that the plates were so heavy that the colonies could not be counted.

hours afterwards the bacteria begin to increase in number and septicemia becomes heavier and heavier until the death of the animal. The rabbits died in from one and a half to three days. Rabbits 2 and 3, especially 3, showed some variations from this rapid, progressive course. Rabbit 3 lived six days and its blood cultures give a possible explanation for this. At seventy-two hours the blood had reached so great a septicemic state that the animal should have succumbed on the following day. But the rabbit lived and the blood culture showed a retrogression of the infection for two days. This was followed by another exacerbation which was also overcome and succeeded by another retrogression. The rabbit died on the sixth day with a low culture; but six hours elapsed between the last bleeding and the death of the animal. This gave sufficient time for another ascension, which probably occurred, and as a result of which the rabbit died. Such fluctuations or crises cannot be ascribed to the technique, since they were often observed, and also a temporary reduction in the number of bacteria was always accompanied by a prolongation of the rabbit's life. Infections running such zigzag courses are positive indications that a two-sided warfare occurs within the body of the host; the bacteria make advances which are successfully met by the defensive power of the rabbit, but, not being entirely killed out, they make another trial. Such a struggle may be prolonged for several days, and the final results depend upon the relative strengths of the aggressive forces of the invading bacteria and the defensive resources of the infected animal.

Experiment 3.—This experiment was carried out two months after experiment 1. The same strain of streptococcus was used in this experiment and the same quantities of bouillon culture were injected. This strain had been kept on artificial media continuously and had evidently suffered a loss of virulence. The technique was the same as in experiment 1. Two typical rabbits are reported in table III.

The courses of the infections reported in table III show that the bacteria had lost in aggressiveness but they still possessed an insusceptibility to the destructive influences of the host and were yet able to carry on a local fight in some more or less inaccessible place. The local lesions probably lowered the vitality of the animals to

TABLE III.

Animal.	Time of bleeding and number of colonies.											
Rabbit 1, weight 1,540 gm. Autopsy.	30 min.	2 hrs.	6 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	140 hrs.	144 hrs.	168 hrs.	192 hrs., rabbit died.
	76	0	0	0	0	0	0	0	0	0	150	
	Pleurisy with effusion. Streptococci abundant in the fluid. Smear from the heart's blood heavily positive.											
Rabbit 2, weight 1,720 gm. Autopsy.	30 min.	5 hrs.	12 hrs.	24 hrs.	36 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.	168 hrs.	192 hrs.
	30	1	80	50	30	30	25	0	10	50	200	×
	216 hrs., rabbit died. Pericarditis, and extensive cellulitis over the abdomen. Smears from these lesions were heavily loaded with streptococci.											

such an extent that the bacteria were able to produce a general infection. Or, on the other hand, the life within the tissues of the host increased the infectivity of the bacteria and they were able to overcome the opposition offered by the rabbit.

Experiment 4.—This experiment was made one month after experiment 3. The bacteria had been kept on artificial media during this time. The quantities of culture used and other points of technique were the same as in the preceding experiments. Three rabbits are given in table IV to represent the nature of infections obtained at this time.

In experiment 4 the bacteria were almost devoid of infecting power and they behaved very much as saprophytes; they rapidly disappeared from the circulation and were not able to reappear. The rabbits showed no signs of disease and remained in perfect condition.

PNEUMOCOCCUS INFECTIONS.

A number of experiments were carried out with the pneumococcus similar to those just described with the streptococcus. Depending upon the virulence and the number of bacteria injected, a pneumococcic infection in the rabbit may take any one of the three courses described in the streptococcic infections. The initial decrease in the number of bacteria in the blood may not be as complete as with the streptococcus, especially if a very large number of bacteria are injected. Pneumococci usually begin to reappear in the blood earlier than streptococci. The infections are more acute and severe. The types of infection are even more defined than with the streptococcus. The acute infections last from two to three days. In from two to three hours the bacteria have largely disappeared from the blood. Cultures taken at five hours usually show an increase in the number of colonies and this reaches a heavy septicemia in from eighteen to twenty-four hours. The animals die within forty-eight to seventy-two hours. Rabbit 1 in table V is an example of this type of infection. If the bacteria have lost in virulence or if a smaller number is injected, a chronic infection occurs. Rabbit 2 of table V represents this class typically. The bacteria disappear from the blood more rapidly and the reappearance is delayed and the increase is slight and is followed by a second decrease. The rabbits usually have a low septicemia for several days. This is followed by a rise and the animal dies within one to two

TABLE IV.

Animal.	Time of bleeding and number of colonies.							
Rabbit 1, weight 1,380 gm.	30 min. 75	2 hrs. 4	20 hrs. 3	26 hrs. 5	44 hrs. 20	66 hrs. 0	72 hrs. 0	The bacteria never reappeared in the blood and the rabbit was in perfect condition 1 mo. later.
Rabbit 2, weight 1,620 gm.	10 min. 180	4 hrs. 0	20 hrs. 0	The blood remained sterile and the rabbit was in good condition after 1 mo.				
Rabbit 3, weight 1,200 gm.	3 hrs. 7	20 hrs. 0	44 hrs. 12	64 hrs. 0	112 hrs. 0	Bacteria never reappeared in the blood, and the rabbit continued well.		

days. At autopsy severe local lesions are always found. The localization may be in the pericardium, pleura, peritoneum, or in the subcutaneous tissues of the abdomen. In one rabbit localization occurred in the kidneys. Any two or more of these places may be involved in the same animal, but many times only one is affected. The bacteria accumulate in great quantities in these localities; when present, septicemia almost invariably precedes death. In the third type the rabbit masters the situation within a few hours; the bacteria disappear from the blood permanently and the animal continues in perfect condition. Rabbit 3 in table V falls in this class.

SUMMARY.

When rabbits are injected intravenously with a quantity of virulent streptococci or pneumococci sufficient to cause death within two to four days the septicemia takes a definite course with slight variations. The bacteria rapidly decrease in number from the time of the injection to from two to four hours, at which time the blood is sterile or contains only a few bacteria. Within five to six hours the bacteria reappear in the blood and steadily increase until the death of the animal. If the bacteria are less virulent, the same quantity of culture causes a chronic type of infection. The same initial decrease in the number of bacteria occurs. The reëntrance into the blood is somewhat delayed, the septicemia does not reach the height obtained in the acute cases, and a second fall occurs within the course of a few hours. These rabbits show a low blood invasion or a sterile blood culture for several days. During this time they become emaciated to a marked degree. Then the low septicemia rapidly rises or the rabbit with a sterile culture develops a severe septicemia within a few hours and death takes place from a few hours to two days thereafter. In this type of infection local lesions, pericarditis, pleurisy, peritonitis, etc., are usually found. In the infections which run an acute course no gross lesions are found. If the bacteria are still less virulent they never reënter the blood after the initial disappearance and the rabbits remain in good condition. In order to obtain uniform results, the quantity of bacteria injected must not be so large that the bacterial substances

TABLE V.

Animal.	Time of bleeding and number of colonies.										
Rabbit 1,	30 min.	3½ hrs.	22 hrs.	27 hrs.	In 44 hrs. rabbit was found dead.						
weight 1,450 gm.	150	0	×	×							
Rabbit 2,	5 min.	30 min.	1½ hrs.	3 hrs.	4½ hrs.	7 hrs.	11 hrs.	21 hrs.	25 hrs.	28 hrs.	48 hrs.
weight 1,700 gm.	350	3	0	0	3	15	1	100	350	200	125
	72 hrs.	96 hrs.	Dead 120 hrs. Autopsy showed pleurisy, pericarditis, and cellulitis over the ab-								
	100	200	domen. Films from these places were heavily loaded with pneumococci.								
Rabbit 3,	10 min.	4 hrs.	20 hrs. The blood remained sterile and the rabbit fully recovered.								
weight 1,620 gm.	180	0	0								

carried in are sufficient to cause an intoxication of the animal. If the quantity of bacteria injected is below this point the course of the infection depends largely upon the virulence of the infecting organisms. Yet variations in the natural resistance of individual animals may be sufficient to cause quite marked irregularities in the course of the infection. Pneumococci can be standardized so as to produce a particular type of infection more easily than streptococci. In general infections such as those produced by streptococci and pneumococci the number of the bacteria present in the circulating blood at a given time supplies accurate and delicate information regarding the severity of the disease. When the object is to determine the degree of virulence of bacteria, or of the efficiency of an experimental therapeutic method, the mere physical condition and mere death of the inoculated animals are not sufficient and satisfactory guides to the desired information. The death of the inoculated animal and the recovery of the infecting bacteria at autopsy do not give complete information concerning the intensity and course of the infection occurring during life. A large number of bacteria found in the blood and tissues at autopsy do not necessarily prove the existence of a heavy infection before the onset of the death agony, since it is a well known fact that bacteria multiply with enormous rapidity, once the natural resistance of the animal has been overcome. Therefore, if merely the life and death of the animal and autopsy findings must serve as our only guides, we shall lose much incidental information, perhaps of fundamental value. This may be especially true as regards the search for curative substances. Again, the individual animals of the same species, age, and apparently of identical physical condition react to the aggressive force of the infecting organisms variously. This fact is readily found out by the injection of a series of rabbits with lethal quantities of bacteria per body-weight, and by making tests at various periods before death results, which, in the case of streptococci, ranges from one to six days. Consequently a method which enables the determination of the degree and progress of the infection at any desired period is of obvious advantage.

LOCALIZATION OF THE VIRUS AND PATHOGENESIS OF EPIDEMIC POLIOMYELITIS.*

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Since epidemic poliomyelitis is an affection chiefly of the central nervous tissues, it may be supposed that the nervous organs possess a special affinity for its microbic cause.[1] Once the virus of the disease has gained access to, and multiplied within, the nervous tissues, it survives there, apparently, longer than it does in other organs in which, under ordinary conditions, it occurs far less regularly. It has been shown by experiment that the poliomyelitic virus readily reaches the central nervous system when it is brought into relation with the peripheral nerves. It is in this manner that infection is induced when the virus is brought into contact with the nasal mucosa, sciatic nerves, and probably also when it is injected into the subcutaneous tissues and peritoneal cavity.[2] Thus deposited, the virus ascends by way of the nerves to the olfactory lobes of the brain or to the spinal cord and intervertebral ganglia. It is to be assumed that in the case of the spinal nerves the ascent is by way of the afferent or sensory fibers; in the case of the nasal membrane, along the olfactory fibers. Hence the virus is carried both by nerves of common and of special sensation. In these instances the virus is brought into relation, not with special end organs, but with nerve fibrils, along which it travels. It appears, however, that the virus may enter the nerves by way of specialized end organs, which are themselves not appreciably injured by it.

INTRAOCULAR INOCULATION.

The demonstration that the virus of poliomyelitis may penetrate the uninjured sensory end organs has been made in connection with the optic nerve. An emulsion of the spinal cord carrying the active

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[1] Flexner, S., *Jour. Am. Med. Assn.*, 1910, lv, 1105.

[2] Flexner, S., and Amoss, H. L., *Jour. Exper. Med.*, 1914, xix, 411.

virus and free from bacterial contamination can be injected into the vitreous chamber of the eye without causing appreciable inflammation. A small quantity of the vitreous humor is aspirated, under ether anesthesia, by means of a tuberculin syringe carrying a very fine needle, and replaced by an emulsion of the virus. The cloudiness thus caused tends to disappear, and the vitreous body to return to normal. After a variable incubation period, symptoms of experimental poliomyelitis appear, paralysis develops, and the pathological findings are characteristic of the disease.

Experiment 1.—Macacus rhesus. May 29. Withdrew under ether anesthesia as stated 0.1 c.c. vitreous humor by puncture of sclera and introduced 0.2 c.c. emulsion of active virus. June 7. Excitable. June 9. Weakness of arms. June 10. Ataxic. June 12. Paralysis of right arm; weakness of left leg; both eyes normal. June 15. Arms and legs weak. From this date the condition remained stationary until June 22, when death occurred from diarrhea.

The autopsy revealed old and recent dysenteric lesions of the large intestine. No visible changes were observed in the spinal cord or eyes. However, microscopical examination of the spinal cord, medulla, and intervertebral ganglia shows typical lesions of poliomyelitis. The retina of the inoculated eye appears normal.

Experiment 2.—Macacus rhesus. June 12. Withdrew, in the manner of experiment 1, 0.1 c.c. vitreous humor from the left eye and injected 0.2 c.c. paper filtrate of an emulsion of virus. The cloudiness of the vitreous humor did not entirely clear up. June 19. Excitable. June 22. Tremor of head; weakness of left leg; ataxia. June 23. Paralysis of left arm; weakness of right arm; double ptosis and slight left facial paralysis; weakness of left leg. June 24. Prostrate; etherized.

The spinal cord, medulla, and intervertebral ganglia present typical lesions of poliomyelitis. The ophthalmic ganglion on the inoculated side shows also a diffuse cellular infiltration.

The two experiments given confirm the supposed affinity which the poliomyelitic virus possesses for nervous tissues and they indicate also that the virus is capable of penetrating highly specialized end organs in order to reach peripheral nerves, along which it penetrates to the central nervous system. Incidentally it shows that the virus may pass from peripheral nerves into the adjacent ophthalmic ganglion, but whether directly from the eye, or indirectly after infection of the central nervous organs as occurs with the Gasserian ganglia, does not appear.[3]

[3] Landsteiner, K., and Levaditi, C. (*Compt. rend. Soc. de biol.*, 1909, lxvii, 787) succeeded in one instance in producing paralysis by inoculating the virus into the anterior chamber of the eye.

DISTRIBUTION OF THE VIRUS AFTER INTRAVENOUS INOCULATION.

It is established that experimental poliomyelitis may be caused with more or less regularity by insuring that the virus reaches the central nervous organs by way of the peripheral nerves. When the virus is brought directly into relation with the central nervous system by intracerebral and intraspinous injections the most constant results are secured. Probably the less constant effects which follow injection of the virus into the peripheral nerves result from the fact that the greater the distance the virus is compelled to travel along nerves, the more chances there are for miscarriage of infection, either by reason of too great dilution, or failure of the virus to reach the central organs at all.

In all these instances the affinity of the nervous organs for the virus may be exerted directly, since the virus is brought either immediately into relation with the nervous tissues, or reaches them directly through lymphatic communication. When the virus is brought to the nervous organs by means of the blood, it is at first separated from the tissues themselves by the vessels and other structures interposed between the blood itself and the nervous tissue. For this reason it has been observed that, while small or even infinitesimal doses of the virus suffice to induce quite constant infection by the intranervous mode of inoculation, large quantities of the virus produce only occasional and inconstant infection, when injected directly into the blood.

The cause of this discrepancy has already been traced to an apparent inability of the virus to enter directly the substance of the brain and spinal cord from the blood.[4] In order to reach these organs, the virus must, it appears, leave the blood and pass into the cerebrospinal fluid, with which it reaches the interstices of the tissues. Since the cerebrospinal liquid is a product of the activity of the choroid plexus, it has been assumed that the virus must first penetrate that structure. Experiments have been performed in order to study this phase of the subject more closely.

Infectivity of Organs.—When the virus is injected directly into the blood it is quickly distributed throughout the circulatory system

[4] Flexner and Amoss, *loc. cit.*

in the manner in which other microörganisms are distributed. In due time it may be assumed that the virus is deposited in certain organs, since experiment has shown that it does not remain long in the circulating blood.[5] Hence it is readily possible to ascertain the distribution of the virus by sacrificing at intervals the infected animals, and inoculating emulsions of the organs themselves. In this manner it can be determined whether the virus is distributed mechanically, or according to the affinity which the several organs display toward it.

The next experiments to be described have been devised to answer this question.

Experiment 3.—Macacus rhesus. May 4. 250 c.c. of a centrifugalized suspension of the spinal cord and medulla containing the active virus were injected into one of the superficial veins of the leg. Three days later, on May 7, the animal was etherized and the spinal cord, medulla, crura cerebri, and intervertebral ganglia were removed aseptically. These were made into 5 per cent. suspensions which were injected intracerebrally into three *Macacus rhesus* monkeys, A, B, and C.

Monkey A.—May 7. 2 c.c. of emulsion of intervertebral ganglia injected. May 10. Excitable. May 12. Right arm weak; left arm paralyzed. May 13. Prostrate; etherized. Typical poliomyelitis.

Monkey B.—May 7. Injected 2 c.c. of emulsion of spinal cord and medulla. This animal developed no symptoms and remained well indefinitely.

Monkey C.—May 7. Injected 2 c.c. of emulsion of crura cerebri. No symptoms of poliomyelitis developed and the animal remained well indefinitely.

Experiment 4.—Macacus rhesus. Apr. 16. 240 c.c. of centrifugalized virus were injected intravenously. Four days later, Apr. 20, the animal was etherized. There were removed aseptically for inoculation: spleen, bone marrow, kidneys, spinal cord and medulla, which were made into 5 per cent. emulsions and injected intracerebrally into *Macacus rhesus* monkeys, D, E, F, and G.

Monkey D.—Apr. 21. Received 2 c.c. of the suspension made from portions of spinal cord and medulla. No symptoms of poliomyelitis developed, and the animal remained normal.

Monkey E.—Apr. 21. Received 2 c.c. of a suspension of portions of each kidney. No symptoms of poliomyelitis developed, and the animal remained normal.

Monkey F.—Apr. 21. Received 2 c.c. of a suspension of the spleen. Apr. 21. Excitable; ataxic. Apr. 25. Both legs and right arm paralyzed. Apr. 27. Died. Typical poliomyelitis.

Monkey G.—Apr. 21. Received 2 c.c. of suspension of bone marrow of both femurs. Apr. 24. Excitable. Apr. 29. Tremor; weakness of neck. Apr. 30. Prostrate; etherized. Typical poliomyelitis.

[5] Clark, P. F., Fraser, F. R., and Amoss, H. L., *Jour. Exper. Med.*, 1914, xix, 223.

Experiment 5.—Macacus rhesus. Mar. 31. Intravenous injection of 250 c.c. of centrifugalized virus. Five days later, Apr. 5, etherized and portion of spleen, bone marrow, spinal cord and medulla were removed aseptically and made into 5 per cent. emulsions and injected intracerebrally into *Macacus rhesus* monkeys, H, I, and J.

Monkey H.—Apr. 5. Injected 2 c.c. of suspension of spinal cord and medulla. No symptoms developed and the animal remained normal.

Monkey I.—Apr. 5. Injected 3 c.c. of the suspension of the spleen. Apr. 8 Excitable. Apr. 11. Ataxic. Apr. 12. Tremor; prostrate. Apr. 13. Died. Typical poliomyelitis.

Monkey J.—Apr. 5. Injected 3 c.c. of suspension of bone marrow from both femurs. Apr. 10. Excitable; ataxic; weakness of left leg. Apr. 17. Legs and left arm paralyzed; prostrate; etherized. Typical poliomyelitis.

The sections of the central nervous organs show not only marked lesions of poliomyelitis, but those of the fourth ventricle reveal an infiltration of the choroid plexus with mononuclear cells.

This series of experiments is consistent in exhibiting that, in spite of the essential affinity which the spinal cord and brain exhibit for the poliomyelitic virus, they are, nevertheless, unable to remove it directly from the blood, prior to some change taking place in the structures (vascular or secretory) that preside over the production of the cerebrospinal fluid; while the spleen and bone marrow, but not the kidney, readily remove it from this source. The fact that the intervertebral ganglia are capable of readily removing part of the virus from the blood shows, first that their relation to the blood vessels differs from that of the brain and spinal cord, and second explains the constant and early involvement of these structures in the poliomyelitic process. The ganglia, therefore, appear capable of obtaining the virus from two sources, namely, directly from the blood, and indirectly from the cerebrospinal fluid.

Moreover, the poliomyelitic virus may be retained alive in the body for a considerable period of time without gaining access to the interior of the central nervous organs.

Experiment 6.—Macacus rhesus. Apr. 18. Injected intravenously 250 c.c. of centrifugalized virus. No symptoms of poliomyelitis developed and seventeen days later, May 5, the animal was etherized. 5 per cent. suspensions of spleen and central nervous tissues were prepared and inoculated intracerebrally in monkeys K and L. Microscopic examination of sections of the spinal cord, medulla, and intervertebral ganglia prove them to be free from lesions of poliomyelitis.

Monkey K.—May 6. 2 c.c. of emulsion of medulla and spinal cord injected. No symptoms developed, and the animal remained normal.

Monkey L.—May 6. 2 c.c. of suspension of spleen injected. May 12. Excitable; ataxic. May 16. Arms weak; etherized. Poliomyelitis.

This experiment indicates, first that the virus of poliomyelitis is capable of surviving for a considerable period in the interior of the body, without inducing an infection of the central nervous system; and next that this long sojourn is not without effect on the quality of the virus, which would appear to have been weakened by the action of the spleen.

Effect of Aseptic Meningitis.—The permeability of the meninges for the contents of the blood is increased by inflammation of those structures. The introduction of sterile alien blood serum into the subarachnoid spaces causes an aseptic inflammation of mild degree that reaches its maximum in twenty-four hours, and then subsides. The inflammation is marked by emigration into the pia-arachnoid, cerebral ventricles, and choroid plexus of polymorphonuclear leucocytes chiefly, and by the escape of plasma. Neither the ependymal epithelium nor the perivascular lymphatics show appreciable change.

Experiments were conducted to ascertain the effect of this inflammation on the penetration of the virus of poliomyelitis into the central nervous organs.

Experiment 7.—Control. *Macacus rhesus.* Apr. 23. Injected intravenously 25 c.c. of centrifugalized suspension of spinal cord and medulla of paralyzed monkey. No symptoms developed and the animal remained normal.

Experiment 8.—Control. *Macacus rhesus.* Apr. 23. 50 c.c. of the centrifugalized suspension of virus used in the previous experiment were injected intravenously. The animal remained normal.

Experiment 9.—Macacus rhesus. Apr. 22. Injected intraspinously 3 c.c. of 40 per cent. inactivated horse serum. Apr. 23. Lumbar puncture; 0.5 c.c. of turbid fluid containing large numbers of white corpuscles was obtained. Injected intravenously 25 c.c. of centrifugalized suspension of virus as in experiments 7 and 8. Apr. 26. Excitable; legs weak. Apr. 30. Arms also weak; etherized.

Microscopic examination of the medulla, spinal cord, and intervertebral ganglia reveals pronounced lesions of poliomyelitis. While vascular lesions are everywhere pronounced, lesions of the nerve cells, interstitial substance, and meninges are also marked. The choroid plexus of the fourth and lateral ventricles is included in the sections. The plexus of the fourth ventricle shows definite infiltration with mononuclear (lymphoid) cells; the plexus of the lateral ventricle is less infiltrated. The blood vessels in the floor of the fourth ventricle are heavily infiltrated, while the deeper vessels are less affected, and those beneath the lateral ventricle are unaffected.

Experiment 10.—Macacus rhesus. May 5. Injected intraspinously 3 c.c. of 40 per cent. inactivated horse serum. May 6. Injected intravenously 25 c.c. of centrifugalized suspension of active virus. May 9. Excitable. May 13. Right arm paralyzed. May 14. Died.

The microscopic examination of sections of the medulla, spinal cord, and ganglia shows poliomyelitic lesions of moderate degree. The most pronounced lesions occur in the floor of the fourth ventricle. Vascular lesions are nowhere severe.

The foregoing observations raise anew the question as to the path traversed by the virus from the blood to the cerebrospinal fluid, and thence to the nervous tissue. Since the cerebrospinal liquid is the product of the secretory activity of the choroid plexus, it has been assumed that the impermeability of the plexus for most foreign products results in the exclusion also of microörganisms, as long as the secreting structures remain intact. Apparently such moderate quantities of the poliomyelitic virus as are contained in twenty-five to fifty cubic centimeters of a clear suspension of the spinal cord and medulla, taken from a paralyzed monkey, may be insufficient to inflict the necessary damage upon the choroid plexus, while still larger quantities may suffice to accomplish this. When, however, the superficial structures of the nervous organs, such as the meninges and choroid plexus, are put into a state of mild chemical inflammation, their permeability is increased, so that what was before an inadequate quantity of virus is now rendered sufficient to cause poliomyelitic infection.

Another fact has emerged from these experiments: while the blood vessels of the spinal cord and brain may show no unusual degree of pathological alterations, definite lesions of an infiltrative nature may appear in the choroid plexus itself. Before, however, a decision is reached as to the relation of the histological lesions to the escape of the virus from the blood, it is desirable to determine the nature of the lesions in animals that have developed paralysis from large unaided injections of the virus, administered intravenously.

Lesions Caused by Intravenous Injections.—While rhesus monkeys show almost no difference in susceptibility to the action of the poliomyelitic virus when introduced directly into the brain, they exhibit distinct differences when injections are made into the peripheral

parts of the nervous system, or into the blood. Moreover, the quality of the virus itself is brought out by the site of inoculation, since a specimen that is of less than maximal activity may be infectious even in minute doses, when introduced into the brain, and either not active at all, or slightly infectious when injected into the blood, or even into the sciatic nerves. As the experiments that follow show, the quantity of the centrifugalized suspension carrying the virus required to cause infection by the unaided blood route usually exceeds fifty cubic centimeters, while even 200 or more sometimes fail, although a few tenths of a cubic centimeter of the same virus succeed when introduced into the brain.

Before describing the lesions present in the spinal cord, brain, and intervertebral ganglia, caused by intravenous injections, it is desirable to present briefly the nature of the lesions of experimental poliomyelitis such as result from other modes of inoculation. The several lesions may be considered as they affect first the meninges, second the spinal cord, third the medulla and pons, fourth the cerebrum, and fifth the ganglia.[6]

The meninges of the cord and medulla show, as a rule, mononuclear cellular infiltration most pronounced adjacent to, or surrounding, the blood vessels which enter the fissures of the cord and are present in the floor of the fourth ventricle. The general infiltration of the pia-arachnoid is interstitial and as a rule not heavy, while the invasion about the vessels within the perivascular lymphatics is usually heavy, and sometimes is nodular. The spinal cord presents lesions most pronounced in the anterior gray matter, less marked in the posterior gray matter, and least present in the white matter. They are perivascular, interstitial, and parenchymatous. The vascular lesions, which are often pronounced, extend inward from the meninges; the interstitial ones are associated with the presence of mononuclear, to a less extent of polynuclear cells, rarely of red corpuscles, and commonly of serum. Actual necrosis of the ground substance arises, but is uncommon on a large scale. The anterior gray matter is rarely wholly destroyed at certain levels. The interstitial lesions can, in some instances, be traced outwards, directly from affected vessels. The lesions of the parenchyma consist of degeneration and necrosis of ganglion cells, occurring chiefly but not exclusively in the anterior gray matter. The necrotic cells are commonly invaded by phagocytes, the so called neurophages. It is not usual for definite relation to be obvious between the altered blood vessels and the affected interstitial substance or parenchyma. The lesions of the medulla resemble those of the spinal cord, except as they are modified by differences in structure. The vessels most infiltrated are those present im-

[6] Flexner, S., and Lewis, P. A., *Jour. Exper. Med.*, 1910, xii, 227.

mediately beneath the fourth ventricle; the deeper lying vessels tend to be less affected, and the very small branches throughout the part are involved inconstantly. The focal interstitial lesions tend to be smaller than those of the cord. No definite relation can, as a rule, be made out between the vascular and interstitial changes. Because of the smaller size and less uniform distribution of nerve cells, the parenchymatous lesions are less conspicuous; they are, however, essentially identical with those of the cord. Lesions similar to those in the medulla occur in the pons and crura cerebri, but less frequently. The cerebrum is affected far less constantly than other parts of the nervous system. When present, the lesions are perivascular and focal interstitial. The cerebral meninges, as a rule, escape affection. The choroid plexus of the lateral and fourth ventricles has not been studied in all instances. When the virus has been introduced into the brain, cerebrospinal fluid, or nerves, the rule appears to be that the plexus escapes. However, exceptions to this rule occur, in which case lesions similar to those to be described as occurring after intravenous injection of the virus may arise (compare experiment 5, monkey J). The intervertebral ganglia are invariably affected. The lesions are of two main kinds, interstitial and parenchymatous, and are always focal. The cellular invasion proceeds from two sources; the pial investment and the blood vessels. In the former, direct extension may take place from the spinal meninges, or extension may occur by way of the connective tissue of the nerve roots. In the latter, extension seems to proceed from the blood vessels. It remains, however, to state that the involvement of the blood vessels may not arise through the general blood, but through inclusion of the vessels in the infiltrative process within the septa of the nerve roots. In rare instances the blood vessels present, as compared with other parts of the ganglia, an unusual degree of surrounding infiltration. The ganglionic nerve cells are destroyed in two ways: first, they are obliterated by focal accretions of mononuclear cells, and, second, by necrosis and neurophagocytosis, in the same manner as in the corresponding condition in the spinal cord.

With this description before us, we may now proceed to describe the lesions which arise as a result of infection by the intravenous mode of inoculation.

Experiment 11.—Macacus rhesus. Nov. 14. 100 c.c. of a Berkefeld filtrate of the virus were injected into the right saphenous vein. Nov. 24. Excitable; paralysis of the left leg and left side of the face. Nov. 25. Paralysis of arms and legs; weakness of back. Nov. 26. Prostrate. Dec. 1. Died.

The spinal cord, medulla, and ganglia show pronounced lesions. Those of the spinal cord affect the blood vessels, interstitial tissue, and nerve cells. They are not distinctive, and do not differ from the usual lesions. This fact is also true of the lesions in the medulla, which are most pronounced in the blood vessels in the floor of the fourth ventricle, and of the ganglia, where they are wide-spread.

Experiment 12.—Macacus rhesus. Nov. 7. Injected intravenously 54 c.c. of a Berkefeld filtrate of the virus. At the same time, under ether anesthesia, 5 c.c. of salt solution were introduced into the left cerebral hemisphere. Nov 16. Excitable; ataxic. Nov. 17. Arms paralyzed; back weak. Nov. 18. Prostrate. Nov. 19. Etherized.

The lesions of the spinal cord, medulla, ganglia, and cerebrum are pronounced. Not only are the usual lesions present, but in addition severe affection of the blood vessels in the cord, medulla, and cerebrum occurs, from which infiltrations extend into the substance of the nervous tissues. The cerebral meninges at the site of the injection of salt solution also show infiltration.

Experiment 13.—Macacus rhesus. Jan. 30. 250 c.c. of centrifugalized suspension injected intravenously. Feb. 14. Excitable. Feb. 16. Weakness of legs. Feb. 17. Paralysis of arms. Feb. 18. Weakness of back. Feb. 19. Died.

The lesions are typical of poliomyelitis. The meninges of the spinal cord are diffusely infiltrated. The vascular lesions are moderate. There is widespread degeneration of the nerve cells, but none of the lesions are distinctive, or differ from those usually occurring.

Experiment 14.—Macacus rhesus. Oct. 30. Intravenous injection of 180 c.c. of centrifugalized virus. Nov. 9. The animal shows weakness and disturbance of vision. During the night it died.

The medulla, spinal cord, and ganglia are the seat of marked lesions of poliomyelitis. The vascular infiltration is heavy but the usual larger vessels only are affected. The cerebrum is devoid of lesions, while the crura cerebri are the seat of interstitial, but not of striking vascular lesions.

Experiment 15.—Macacus rhesus. Feb. 28. Intravenous injection of 250 c.c. of centrifugalized virus. Mar. 4. Excitable. Mar. 5. Arms and legs weak; ataxic. Mar. 6. Died.

The lesions of the spinal cord are perivascular, interstitial, and meningeal; of the ganglia, perivascular, with extension into the nerve roots. The medulla shows a high degree of affection of the blood vessels. The cerebrum has escaped, but the choroid plexus of the lateral ventricle, but not of the fourth ventricle, shows edema and perivascular cellular infiltration. The ependymal cells appear normal.

Experiment 16.—Macacus rhesus. Apr. 16. Intravenous injection of 250 c.c. of centrifugalized virus. During the injection a needle was kept in the lumbar spinal canal. Apr. 21. In the morning the arms and back were paralyzed. In the afternoon death occurred.

The lesions in this instance are very pronounced. The blood vessels within the spinal cord, medulla, and pons show wide involvement, while the interstitial tissue and nerve cells are affected only moderately. The cerebrum is devoid of lesions, while the choroid plexus of the lateral ventricles contains a slight accumulation of mononuclear cells about the blood vessels. The plexus of the fourth ventricle appears normal.

Experiment 17.—Macacus rhesus. Apr. 16. 240 c.c. of centrifugalized virus injected intravenously. Apr. 20. Lumbar puncture yielded a fluid containing an excess of white corpuscles. Apr. 21. Excitable; no paralysis; etherized.

The spinal cord, medulla, and pons show early vascular, but no other lesions. The ganglia, however, contain focal cellular infiltrations of small size, and a small number of single necrotic nerve cells. No changes were detected in the choroid plexus.

Experiment 18.—Macacus rhesus. Apr. 16. 240 c.c. of centrifugalized virus injected intravenously. Apr. 20. No symptoms appeared; etherized.

No lesions were detected in the spinal cord, medulla, or choroid plexus, while the ganglia show early infiltrative lesions about the blood vessels, and a few instances of necrosis of single nerve cells.

We may consider this series of experiments according as the lesions affect the nervous tissues proper, or as they affect the choroid plexus.

Within the nervous tissues proper, the lesions are, at times, precisely similar to, and indistinguishable from, those produced by intraneural modes of inoculation. However, in certain instances, the lesions present not only resemble those caused by the intraneural modes of inoculation, but differ from them in the extent and degree to which the blood vessels, and those especially in the medulla and pons, are affected. While the degree of perivascular infiltration does not afford a basis of discrimination, a sharp distinction may be drawn between the usual degree of vascular involvement, and the unusual extent in which it occurred in several cases of intravenous injection. What is especially impressive in the latter instances is the diffuse participation of small vessels, down to those of capillary size, in the process and the extension of the infiltrative process from them to the surrounding nervous tissues. Vessels so greatly altered as those under consideration may be considered as contributing to the permeation of the virus from the blood into the tissues. The early lesions of the intervertebral ganglia should be emphasized in this place since they antedate those of the spinal cord and medulla, and extend apparently from vascular lesions.

What appear, however, to be especially important are the changes detected in the choroid plexus, in which infiltrative lesions have hitherto not been observed. That definite lesions of the plexus may occur is clearly indicated by the experiments. As yet no evidence has been obtained of morphological alterations in the ependymal cells, but merely in the blood vessels beneath them. That the secretory functions of the plexus are altered in the direction of greater permeability may be safely assumed, from which it follows that the experiments indicate that when the poliomyelitic infection is induced by the intravenous injection of the virus, there arise, not only the common lesions of poliomyelitis, but also certain additional lesions of the blood vessels and choroid plexus which are of peculiar and distinctive nature.

Since the precise mode of infection in human cases of poliomyelitis may be regarded still as an open question, this criterion of a blood invasion may prove of assistance in the solution of the problem. So far as can be judged from the study of the tissues from several human cases, a corresponding wide-spread vascular involvement to that arising in the experiments would seem not to have occurred.

INTRASPINOUS SERUM PROTECTION.

The data presented confirm and extend the observations already made concerning the passage of the poliomyelitic virus in transit to the central nervous organs from the blood to the cerebrospinal fluid. Since the virus is known to pass successively over several days into the cerebrospinal fluid,[7] in which it seems not to accumulate, but from which it is transferred to the nervous tissues, it was thought that the introduction of a potent immune serum into the meninges at intervals over a number of days would suffice to neutralize the translated virus and thus prevent infection. For this purpose immune serum was available from several monkeys which had recovered from poliomyelitis and had been subsequently reinforced by large subcutaneous injections of the virus.

Earlier experiments had shown that the intraspinous injection of an immune serum is effective under circumstances in which the intravenous injection is not, in delaying or preventing poliomyelitic infection in the monkey. For the next series of experiments it was necessary at the outset to insure that the intravenous injections of the virus would alone induce paralysis, which was accomplished by employing the device of setting up an aseptic meningitis with horse serum, in the animals about to be inoculated.

SERUM AFTER INTRAVENOUS INOCULATION.

Experiment 19.—Macacus rhesus. May 26. Intraspinous injection of 3 c.c. of inactivated 40 per cent. horse serum. May 27. Intravenous injection of 50 c.c. of centrifugalized virus, followed immediately afterwards by an intraspinous injection of 3 c.c. of normal monkey serum. The normal serum was injected intraspinously on May 28, 29, and 30, and, after a two days' interval, on June 2, 3, and 4. June 3. Excitable. June 9. Paralysis of arms and back. June 10. Prostrate; etherized. Typical poliomyelitis.

[7] Flexner and Amoss, *loc. cit.*

Experiment 20.—Macacus rhesus. May 29. Intraspinous injection of 3 c.c. of inactivated 40 per cent. horse serum. May 30. Intravenous injection of 50 c.c. of centrifugalized virus, followed immediately afterward by an intraspinous injection of 3 c.c. of immune monkey serum. The immune serum injections were repeated May 31, June 1 and 2, and, after a two days' interval, on June 5, 6, and 7. No symptoms developed, and the animal remained normal.

Since these experiments show that the virus may be neutralized by an immune serum in process of passage by way of the cerebrospinal fluid to the nervous tissues, it seemed desirable to ascertain whether a similar neutralization could be affected in a case in which the virus was introduced directly into the meninges by means of lumbar puncture.

SERUM AFTER INTRASPINOUS INOCULATION.

Experiment 21.—Macacus rhesus. May 27. Intraspinous injection of 1 c.c. of emulsion of virus. Two hours later, intraspinous injection of 3 c.c. of normal monkey serum. The injection of normal serum was repeated on May 28, 29, and 30, and, after a two days' interval, on June 2, 3, and 4. June 3. Excitable. June 7. Ataxia; arms and neck weak. June 8. A.M. Arms and back paralyzed; legs weak. P.M. Died. Typical poliomyelitis.

Experiment 22.—Macacus rhesus. May 27. Intraspinous injection of 1 c.c. of emulsion of virus. Two hours later, injected 3 c.c. of immune monkey serum intraspinously. The immune serum injections were repeated on May 28, 29, and 30, and, after a two days' interval, on June 2, 3, and 4. No symptoms developed and the animal remained well.

The preceding experiments show unmistakably that by introducing an immune serum into the subarachnoid spaces, the poliomyelitic virus is capable of being neutralized within the cerebrospinal fluid into which it is directly introduced, or to which it passes in transit from the blood to the nervous tissues. Probably the neutralization in the latter instance is effected at successive stages in process of transfer of the virus to the central nervous organs. Normal serum lacks this power of neutralization.

It may be considered as highly probable that the neutralization is accomplished before any quantity of the virus becomes attached to the nervous tissues themselves. Earlier experiments had shown that when such minute amounts of the virus as one fiftieth to one tenth of a cubic centimeter are inoculated intracerebrally, neutralization is either wholly impossible to accomplish, or is accomplished with very great difficulty even by intraspinous injections of immune

serum.[8] Hence the experiments described carry a step further the demonstration that the virus introduced into the blood passes by way of the cerebrospinal fluid to the substance of the nervous tissues in those instances in which paralysis results.

RELATION OF CARMIN TO THE CHOROID PLEXUS AND PERIVASCULAR LYMPHATICS.

That the virus of poliomyelitis is capable, in some instances, of passing from the blood to the cerebrospinal fluid may be considered as demonstrated. This passage takes place probably by way of the choroid plexus and possibly also, to some extent, through the blood vessels in the meninges as well as in the substance of the nervous tissues. Although certain lesions have been detected in the choroid plexus, no morphological alterations have been discovered in the ependymal cells themselves. Hence the question arose whether by the use of pigments the ependymal cells in certain pathological states, including poliomyelitis, might be shown to react in a manner supplying ocular evidences of a disturbance of function.

Carmin is a non-toxic pigment which can be sterilized and suspended in a fine state of subdivision. In this form its introduction into the cerebral ventricles and subarachnoid spaces causes no discomfort in monkeys. Its presence in the meninges and ventricles is followed by an inflammation and rich cellular exudation. The emigrated cells are polynuclear chiefly, but mononuclear cells which take up pigment granules emigrate also. The effects of the carmin injections were studied in normal monkeys, and in monkeys in which an aseptic inflammation had been set up twenty-four hours earlier by means of horse serum, or in which poliomyelitis had been induced by intracerebral inoculation of the virus.

A suspension of the pigment was made in 20 per cent. glycerin, and ammonia was added until solution was complete. This solution was autoclaved and immediately before use was slowly neutralized by the repeated addition of small amounts of sterile 2 N hydrochloric acid, until litmus paper indicated change of reaction. The injections were made under ether anesthesia into the lateral ven-

[8] Flexner and Lewis, *Jour. Am. Med. Assn.*, 1910, liv, 1780; 1910, lv, 662.

tricle, the volume of fluid injected being determined by a needle in the lumbar meninges, from which the colored solution was allowed to flow before the injection was stopped.

Within twenty-four hours the pigment is distributed over the surfaces of the spinal cord and brain, and within the cerebral ventricles. The base of the brain is deeply and uniformly pigmented. The intervertebral ganglia are either unaffected or mottled with pigment. The nerve roots are visibly pigmented. The choroid plexus appears a vivid red color.

Normal monkeys etherized respectively twenty hours and five days after the injection of the pigment show the inflammatory reaction of the meninges and ventricles mentioned. Interest centers especially in the relation of the pigment to the ependymal cells, choroid plexus, perivascular lymphatics, and intervertebral ganglia. The differences in this respect between the twenty-hour and the five-day specimens are inconspicuous and unimportant.

The pigment appears in two states of division, namely, as excessively minute particles, smaller than many bacteria, and as coarser grains. The latter are contained largely in the mononuclear cells of the inflammatory exudate. The very fine particles have been taken up by the ependymal cells covering the walls of the ventricles and the surfaces of the choroid plexus. Not all, but many of the ependymal cells contain the pigment particles in varying number. The minute and larger grains occur also within cells in the subependymal layer, in close proximity to the ventricles. The latter pigment-containing cells do not seem to have emigrated from the interior of the ventricles, so it is considered probable that the pigment has passed from the ependymal to the subependymal cells. A small quantity of pigment occurs also in the superficial perivascular spaces in the cortex, but not in the spinal cord. The pigment penetrates to the interior of the ganglia with difficulty, along two courses: first the pia capsular investment, second the septa of the nerve roots. About the pigment there is a marked cellular reaction, and pigment-containing leucocytes come to lie against or near nerve cells, but no wide diffusion occurs within the ganglia. The meninges of the brain and spinal cord show a rich cellular exudation containing pigment.

When an aseptic inflammation has been set up previously, and the animal etherized twenty-four hours after the pigment has been injected, the distribution is identical with that described. The single difference noted is a greater amount of pigment within the ependymal and subependymal cells of the ventricles. In the case of an animal in which the pigment was injected during the early paralytic stage of poliomyelitis and which was etherized twenty-four hours later, the cellular accumulations were greater because of the addition of the polynuclear cells to the usual mononuclear infiltration. The choroid plexus of the lateral ventricle showed marked lymphoid, nodular aggregations beneath the ependymal cells, and a rich leucocytic emigration outside. The quantity of pigment taken up by the ependymal and subependymal cells is somewhat greater than that observed in the other instances.

The experiments with the carmin may be interpreted as indicating that the ependymal cells in a living state can be entered by particulate substances. Whether the strictly normal ependymal cells take up and pass on, in the manner described, pigment particles cannot be deduced from the experiments, as the carmin itself causes an inflammatory reaction and consequently may act injuriously upon the cells. Aside from the presence of the pigment, the ependymal cells exhibit no morphological alteration. Apparently a previous inflammation, such as that caused by horse serum and the virus of poliomyelitis, has the effect of rendering the ependymal cells more permeable for the pigment.

PATHOGENESIS OF POLIOMYELITIS.

A consideration of the experiments described in this paper should deal with the question of the pathogenesis of poliomyelitis which they are believed to elucidate.

We are confronted with the problem as to the site of entrance of the virus of poliomyelitis into the human body, as well as the manner in which the specific lesions of the disease are produced. The latter question has already been cleared up in large measure.[9]

[9] Flexner, *Jour. Am. Med. Assn.*, 1910, lv, 1105. Flexner and Lewis, *Jour. Exper. Med.*, 1910, xii, 227.

It is now sufficiently obvious that the virus possesses affinity for nervous tissues in general, but for no element of these tissues in particular. The constancy with which meninges, blood vessels, interstitial parts, and nerve cells are affected indicates that they all react to the presence of the virus. On the basis of actual observations it cannot be stated that virus is attracted by the nerve cells, either alone or necessarily in advance of the other structures mentioned; while the experiments here recorded show that it is only when the virus is brought to the nervous organs otherwise than by the general blood that the tissues composing them are able readily to remove and attach it to themselves.

This latter fact is a cardinal point, and one from which we may derive valuable information on the pathogenesis and mode of infection of the disease.

It may be regarded as established that all intraneural means of infection are successful, and that the virus travels with more or less ease and certainty along the nerves to the interstices of the central nervous organs, probably utilizing the lymphatic channels of communication. The experiments given in this paper show that the central nervous organs, excepting the intervertebral ganglia, are incapable of removing the virus from the general blood prior to changes induced in the blood vessels and in the choroid plexus. They indicate, also, that in the monkey these preliminary lesions are of a nature that permits of differentiation from the lesions caused by the intraneural modes of infection. The lesions in human cases of poliomyelitis would seem to correspond with those caused by intraneural and not by intravenous inoculation.

In general it should be stated that the intraneural modes of inoculation are effective in proportion to the degree with which they bring the virus into intimate relation with the central nervous tissues. On that account intracerebral inoculation is the most effective, because it not only insures contact between the virus and the mechanically injured tissues, but also because it isolates the virus in the brain tissue, under conditions favorable to multiplication and gradual diffusion into the ventricles and cerebrospinal fluid. Intraspinous injection is somewhat less effective for the reason that a part, and sometimes perhaps all, of the virus may be carried into the gen-

eral blood before it can reach and become attached to the nervous tissues. Intranasal infection is, in keeping with the general statement made above, more certain in its results than subcutaneous or intrasciatic inoculation, because of the proximity of the short olfactory nerve fiber to the brain tissue. It may fail, because the virus is washed away before it reaches the olfactory fibers and can be carried to the brain. Possibly intraocular inoculation may prove among the most successful, because the virus cannot escape and has only a short distance to travel to the brain; while the vitreous humor may even prove a favorable medium for its multiplication.

In the main, under natural conditions, it is the upper respiratory mucous membrane that would most often become contaminated with the virus, and most readily favor its conveyance to the brain. This series of events is determined by the manner in which the virus is thrown off by the infected body,[10] by the fact of its presence upon the nasal mucosa, even in healthy persons in contact with cases of poliomyelitis,[11] and by the demonstration that it passes, on the whole easily, along the olfactory nerve fibers to the brain, medulla, and spinal cord.[12] Although the virus is conveyed to the nervous organs from without by the lymph, the distribution throughout the nervous system is, in large part at least, effected through the medium of the cerebrospinal fluid. Even when the virus passes from the blood into the nervous organs, it takes the indirect course through the cerebrospinal fluid. This important fact has been established, not only by the finding of the virus by inoculation tests in the cerebrospinal fluid[13] after a blood injection, but also through the prevention of infection by the injection of immune serum into the subarachnoid spaces after lumbar puncture following the intravenous infusion of the virus under conditions insuring infection.

[10] Flexner, *Jour. Am. Med. Assn.*, 1910, lv, 1105. Flexner and Lewis, *Jour. Exper. Med., loc. cit.*

[11] Flexner, S., Clark, P. F., and Fraser, F. R., *Jour. Am. Med. Assn.*, 1913, lx, 201.

[12] Flexner and Lewis, *Jour. Am. Med. Assn.*, 1910, liv, 1140. Flexner and Clark, *Proc. Soc. Exper. Biol. and Med.*, 1912–13, x, 1. Flexner, *Lancet*, 1912, ii, 1271; *Science*, 1912, xxxvi, 685. Landsteiner and Levaditi, *Ann. de l'Inst. Pasteur*, 1910, xxiv, 833.

[13] Flexner and Amoss, *loc. cit.*

Thus the experimental evidence, which is upheld by observations in human cases of poliomyelitis, supports the view that epidemic poliomyelitis is caused by the entrance into the body of its specific microbic cause or virus, through the upper respiratory mucous membrane to the olfactory lobes of the brain, from which by means of the cerebrospinal fluid it is distributed throughout the substance of the nervous organs; but, since the virus may reach the brain by way of any nervous channel, and even, although with great difficulty, from the blood, it is, of course, possible that in exceptional instances other modes of infection may arise.

SUMMARY.

The virus of poliomyelitis is capable of penetrating the retina without producing apparent injury, to reach the central nervous organs.

The virus injected into the blood is deposited promptly in the spleen and bone marrow, but not in the kidneys, spinal cord, or brain.

Notwithstanding the affinity which the nervous tissues possess for the virus, it is not removed from the blood by the spinal cord and brain until the choroid plexus and blood vessels have suffered injury.

The intervertebral ganglia remove the virus from the blood earlier than do the spinal cord and brain.

An aseptic inflammation produced by an intraspinous injection of horse serum facilitates and insures the passage of the virus to the central nervous organs, and the production of paralysis. The unaided virus, even when present in large amounts, passes inconstantly from the blood to the substance of the spinal cord and brain.

When the virus within the blood fails to gain access to the central nervous organs, and to set up paralysis, it is destroyed by the body, in course of which destruction it undergoes, as a result of the action of the spleen and, perhaps, other organs, diminution of virulence.

The histological lesions that follow the intravenous injections of the virus in some but not in all cases differ from those which result from intraneural modes of infection.

In escaping from the blood into the spinal cord and brain, the

virus causes a lymphatic invasion of the choroid plexus and widespread perivascular infiltration, and from the latter cellular invasions enter the nervous tissues. A similar lymphoid infiltration of the choroid plexus may arise also from an intracerebral injection of the virus.

The histological lesions present in the central nervous organs in human cases of poliomyelitis correspond to those that arise from the intraneural method of infection in the monkey.

The virus in transit from the blood through the cerebrospinal fluid to the substance of the spinal cord and brain is capable of being neutralized by intraspinous injection of immune serum, whereby the production of paralysis is averted.

Carmin in a sterile and finely divided state introduced into the meninges and ventricles sets up an aseptic inflammation, but is quickly taken up by cells, including ependymal cells. When an aseptic inflammation has been previously established by means of horse serum, or when the nervous tissues are already injured by the poliomyelitic virus, the pigment appears to enter the ependymal cells more freely.

The experiments described support the view that infection in epidemic poliomyelitis in man is local and neural, and by way of the lymphatics, and not general and by way of the blood. Hence they uphold the belief that the *infection atrium* is the upper respiratory mucous membrane.

EXPERIMENTAL TUBERCULOSIS OF THE CORNEA.*[1]

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When an attempt is made to study the tissue reactions against the tubercle bacillus in either the normal or the immune animal, difficulties of a technical character are at once encountered. The final picture in an animal infected with tuberculosis is extremely complicated, and it is impossible to resolve it into its functional elements. Methods of examination during life are practically limited to such general conditions as changes in activity, body-weight, and temperature. The great help derived in the study of other infections from the investigation of changes in either serum or cells of the blood does not touch the problem.

In the belief that it would be of great advantage to be able to study a discrete tuberculous lesion which could at the same time be kept under constant observation without the necessity of resorting, in each experiment, to the tedious technical procedures involved in microscopic examination, we have undertaken a renewed study of experimental corneal tuberculosis.

The work is still in progress. This paper is intended to present primarily the technical phases of the subject, and to indicate briefly the limitations and possibilities of this line of experimentation. Secondarily, there will be presented some facts to show that the corneal lesion is susceptible of influence in an appreciable degree by both local and systemic measures and conditions.

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[1] The experiments here published were used as the basis of a paper read before the Lænnec Society of Johns Hopkins University in April, 1912. Extraneous circumstances have interfered with the prompt publication of the matter. In the meantime the experiments have for the most part been repeated on a large scale in connection with chemotherapeutic studies. The results of the later experiments conform in all essential respects to those here described.

The general features of the lesion of the cornea following inoculation with tubercle bacilli or tuberculous matter were described by Cohnheim and Salomonsen (1) in 1877. Their inoculations were made into the anterior chamber, and consequently their lesion did not develop in detail according to our description.

Other observers have used the corneal experiment to study microscopically the early development of the tubercle, but this is a matter with which we are not here immediately concerned.

Hänsell (2) in 1879 inoculated the cornea, conjunctiva, and anterior chamber with tuberculous matter. He described the corneal lesion in its essentials with accuracy, both in its gross and microscopic features.

Krusius (3) has presented interesting data on experimental tuberculosis of the various parts of the eye. He has developed the fact that the cornea is less susceptible to infection than the anterior chamber and the vitreous humor. It is more susceptible than the lens. Krusius states that the incubation period of the lesion in the cornea is inversely proportional to the dose of culture used. This author notes that in order to get consistent results it is important to use a culture of known virulence and rabbits of equal resistance (same weight, age, etc.). In a general way our results agree with those of Krusius entirely, although we have developed them and have considered the subject from an entirely different point of view. In one or two points there is an apparent contradiction between our results and his. He states that generalization does not occur from the eye lesion even when a culture of bovine type is used. He also found that the lesions showed frequently a distinct tendency to heal. Our animals when they have been kept for a sufficient length of time have always shown tubercles in the internal organs. In but few instances have the corneal lesions shown any definite tendency to heal. These differences indicate that we have used cultures of higher virulence than those employed by Krusius.

The observations on the use of iodin locally have been indicated by the clinical use of this substance more or less regularly since the time of Lugol for the purpose of influencing tuberculosis in one or another of its manifestations.

In using calcium lactate we have followed the observations of Chiari and Januschke (4) on the inhibitory influence of soluble calcium salts administered generally on simple inflammations.

Our observations on benzol have been made under the influence of the experimental work of Selling (5), in which the pronounced leucotoxic action of this substance was developed.

TECHNIQUE.

In all the experiments reported, we have used rabbits. The animals have varied in weight from 1,500 to 3,000 grams, have been of various colors, and purchased from various sources. They were of no especial breed. For most of the experiments we have used a culture of bovine type, isolated from cervical glands in 1907. The culture retains a high degree of virulence, as 0.01 of a milligram intravenously injected will kill a rabbit of 2,000 grams in from

twenty to thirty days, with generalized tuberculosis. The culture was grown on glycerin agar, and the growth was used within fourteen days of the time of transfer. The culture was suspended in a salt solution to make a suspension as homogeneous as possible, and this was centrifuged at high speed for a short time to remove the macroscopic clumps. The suspensions varied in weight, but were, as a rule, made by suspending one glycerin agar tube in ten cubic centimeters of salt solution. The completed preparation was usually of sufficient density to be just completely opaque when contained in the ordinary twenty millimeter bacteriological test-tube.

The eyes before inoculation have been carefully anesthetized with cocain. When this is done, the injection can be made without restraining the animal in any way, and it gives no pain or discomfort. In the beginning we tried to fix the eye with a pair of forceps. We found later that the injection could be made equally well without it. The injury done by the fixation forceps frequently gave rise to secondary foci of infection which should be avoided. We have used a small record syringe, but we have found it better to use special needles that we have had made for the purpose. The most satisfactory are of iridoplatinum, of about gauge 26, and ground to a very short bevel, with a sharp point. The advantage of the platinum needle is that it retains a smooth surface free from rust, even after being sharpened repeatedly. This seems to be very important in making a successful injection. The ordinary steel needles can be used with success when they are new, but they cannot be used after sharpening or repeated boiling.

The injection is made by placing the point of the needle against the cornea in the position desired, holding the syringe in a plane parallel to the cornea, considered as a flat surface. The needle is then inserted by a succession of short, jerky pushes, in which only slightly more force is used than that exerted by allowing the weight of the syringe to bear on the needle point. By this means, the eye is rotated, and between each push is allowed to come back almost to its normal position. When the aperture of the needle is entirely covered by the corneal epithelium, pressure is put on the piston, and the suspension forced into the cornea. Considerable pressure is required. The fluid raises a small vesicle which is at once opaque.

We have not found it practicable to measure the amount of fluid injected, but a fairly uniform series of inoculations can be secured by watching the size of the opacity produced. We have had most uniform results when this measured about three or four millimeters in diameter.

It cannot be said that this technique is perfect. Occasionally, instead of raising a definite circumscribed vesicle, the fluid flows into the cornea along radiating lines, spreading widely from the point of puncture. In a certain number of instances the needle penetrated the anterior chamber. This accident, however, has grown much less frequent as we have gained experience. Moreover, it seems probable that the ultimate result varies, to a certain extent, according to whether the injection mass remains localized close beneath the corneal epithelium, or flows more deeply into the tissue. When the larger amounts suggested are injected, there seems to be less variation due to this factor. In certain instances, instead of injecting the material we have made the inoculation simply by pricking the cornea with the needle wet with the culture. The results of this procedure will be explained later.

In our examinations made subsequent to inoculation we have confined ourselves to the use of oblique illumination. We have found it advantageous to use artificial light because of the greater constancy, and have adopted a 40-watt Tungsten lamp with a ground glass bulb held about sixteen inches distant from the eye. With this we have used a small hand lens.[2]

We have, of course, made careful notes of the development of the lesion, but have found that frequently we save much time and secure, on the whole, more useful records by charting the conditions from day to day.

[2] Dr. Thomas B. Holloway has recently been over the methods of examination of the eye with us and has pointed out the possible usefulness of the corneal loop and the ophthalmoscope. We have not used these instruments extensively, so far as the results at present recorded are concerned, but they will probably add to the accuracy of our examinations in the future. It is likely that the use of these more accurate methods of examination would modify our statements only as they concern the time of appearance of the earliest vascularization of the cornea.

DEVELOPMENT OF THE CORNEAL LESION.

The Effect of Large Doses of Tubercle Bacilli.—When a heavy suspension of tubercle bacilli is injected into the cornea in such a way as to make a distinctly circumscribed infiltrated area of three to four millimeters in diameter, the first result, as has been said, is a circumscribed opacity, which probably marks fairly well the limits of the infiltration. At the end of twenty-four hours this opacity has usually almost disappeared. At this period one is able by careful examination to see the defect in the cornea that marks the point where the needle penetrated, and extending from this defect a very short way into the cornea is a very fine, whitish or brownish white line. At this period, the sclera and bulbar conjunctiva will have their normal appearance, and there will be no evidence of any irritation of the eye. In from three to seven days, depending on various factors, there is a noticeable change. A small increasing opacity of grayish white color develops at the point of inoculation on the cornea, and the conjunctiva of the bulb becomes increasingly injected from day to day. On the seventh day, for the average, the opacity at the point of inoculation has a diameter of about one millimeter. The relation of the size and rapidity of growth of the opacity to the appearance and development of the injection of the bulbar conjunctiva is not perfectly regular, although it is fairly so. As a rule, the conjunctival congestion begins at about the time that the first visible increase in the exudate at the inoculation point shows itself. After several more days, the conjunctival congestion having in the meantime become very intense, loops of newly formed capillaries appear in the cornea, pushing out from the margin towards the inoculation point, and the exudate at the inoculation point continues to increase. The loops of vessels extend further and further into the cornea, until finally they come into contact with the exudate. The congestion of the bulbar conjunctiva becomes complicated by congestion of the conjunctiva of the lids. The lids swell and there is more or less of an increased secretion, which is at times white, at times yellowish. It is usually sufficiently fluid to remove itself spontaneously, but occasionally it becomes sticky and glues the lids together. This does not often occur

before the third week after inoculation. At about the time that the corneal vascularization or pannus comes into contact with the exudate at the point of inoculation, the corneal epithelium over the exudate usually breaks through leaving an open ulcer.

The formation of the pannus takes place in a regular way. The first vessels formed are capillaries and an advancing margin is maintained as a capillary zone. Behind the advancing margin there is a tendency for the pannus to thicken to a definitely raised rather pale zone of granulation tissue. Between this area and the corneoscleral junction the vessels are gradually reduced in number and increased in size until there are formed several main trunks of supply to the more central lesion. These supply trunks run through a cornea which has become partially clear again. In the later stages, with the advance of the exudate and the invasion of the pannus by it, the peripheral clear zone again becomes obscured and filled with granulation tissue.

When large doses are used, the pannus never tends to invade, in any marked degree, the mass of exudation. On the contrary, the exudate gradually extends and infiltrates the pannus. Most of our experiments have terminated at this point. The exceptional instances which have been kept longer under observation will be commented on in later paragraphs in connection with special points. With the large dose in the untreated eye, the vascularization of the cornea usually begins between the eighth and twelfth days.

The Effect of Small Doses of Tubercle Bacilli.—With the results following large doses, we have contrasted the effects of a minimal inoculation. They have been obtained, as before stated, by simply wetting the needle with the culture and pricking the cornea. In making the inoculation in this way it is essential that the epithelium be pierced. If the epithelium be merely superficially scratched, the inoculation is not effective. With the small dose, there come into play the same factors that have been described for the large dose. Very essential differences, however, are to be noted. The rate of progress of the lesion is much less uniform. The ultimate appearance of the lesion, in the majority of cases, is much the same, but in details of the first importance there are variations. It is especially by the use of this method of inoculation that one can deter-

mine differences in individual resistance of the animal. In old rabbits the changes occur much more slowly than in young rabbits. It has occasionally happened, in using this method on old rabbits, that the pannus has completely infiltrated the mass of exudation at the point of inoculation. In the young rabbit it has happened conversely that the pannus has not only been invaded by the mass of exudation, but that it has actually been involved extensively in the ulcer, and has been almost completely eaten away. Various intermediate conditions are encountered.

INFLUENCE OF VIRULENCE OF CULTURE.

We have not so far studied extensively the action of different cultures on the cornea. However, some inoculations were made with cultures of human type which are, of course, non-virulent for rabbits. The large dose before described produced the same end result that the large dose of the virulent culture did. Smaller doses or doses of less virulent human culture produce a lesion which is smaller and which shows a great tendency to heal spontaneously. The lesion in its development follows the general course of that described for the bovine type culture with some differences which serve to place it between the bovine lesion and that about to be described for the non-virulent acid-fast bacilli. The immediate reaction to the human type is somewhat more rapid as a rule. The blood vessels begin to form more quickly but the progress of the lesion is less rapid. While the virulent culture generalizes from the eye as a focus, the human type of culture does not generalize. We hope later to develop this phase of the subject.

CONSEQUENCES OF PERFORATION.

If the cornea is perforated at the time of inoculation or, as has often happened in our series, the ulceration of the cornea occurs internally as well as externally, an infection of the anterior chamber results. The consequences of such infection have been sufficiently described by others. We wish to note, however, one or two points which are of interest by contrast with the pure corneal infection, and which chiefly concern the pannus. The typical pannus, which

is formed as a result of the infection of the cornea, begins at a single point on the corneoscleral junction, extending centrally and broadening out from this point. If the injection is so placed that it is somewhat above central, the pannus always begins at the upper margin of the cornea. When the lesion has become quite extensive, a second pannus may appear starting from below. Placing the injection forward or back of central throws the point of beginning of the pannus either somewhat forward or back, as the case may be. It is an interesting point that when the injection is either precisely central or slightly below central, the first pannus will still form above. From the moment that the cornea perforates internally, however, a circular pannus begins to form. We have controlled this point by injecting the anterior chamber in such a way that the cornea was not injured; that is, by passing the needle through the sclera from behind. Here the pannus is also circular, and no difference between the upper and lower margins can be noted. These differences presumably depend on the circulatory channels in the cornea, but we have at present no more precise explanation for them.

THE EFFECT OF KILLED CULTURES OF THE TUBERCLE BACILLUS.

When we had proceeded with this work for some way, we were led to consider how much of the effect produced by the inoculation was specific for the tubercle bacillus, and how much might depend on the non-specific factors. Before trying anything more remote, we used the same culture killed by heating for one half hour to 60° C. When such a killed culture is injected in about the quantity of a large dose of living culture, the first effect is the same. At the end of twenty-four hours a difference appears, in that the bulbar conjunctiva is usually pronouncedly injected, and the eye appears to be definitely more irritated than by the injection of the living culture. The inoculation point becomes marked out by an exudate in about the same way as with the living culture, but this exudate seldom reaches more than pinhead size. On the fourth or fifth day, as contrasted with the eighth to the twelfth day for the living culture, loops of vessels appear in the cornea. They appear

at the situation favored by the vessels appearing in response to the living culture. They extend very rapidly into the cornea as a thin leash which on the sixth or the seventh day reaches nearly to the point of inoculation. The base of the leash never broadens; it is usually about three to five millimeters in width. From the seventh day on these vessels seem rapidly to disappear. By the tenth day nothing more of them can be seen in the cornea by our methods of examination. No permanent pannus is formed. The exudate at the inoculation site may be entirely reabsorbed, or may persist for months as a pinhead-sized scar. The vessels do not, however, really disappear, at least not within a period of two months. They cease to functionate. This can be shown by making a fresh inoculation with either dead or living cultures near the old scar. Under these circumstances at the time when the congestion of the sclera becomes well marked, the old corneal vessels suddenly fill and the new formation of vessels in consequence of the second inoculation takes place from the end of the old leash.

For obvious reasons we have compared the lesions so far described with the corneal lesion produced by other bacteria. The strains of *Staphylococcus aureus* and *Bacillus typhosus* used, when inoculated on the cornea near its center, produce a rapid local exudation. There is, however, no tendency to vascularize the cornea and the exudate finally resolves more or less completely leaving a relatively small opacity at the inoculation site.

The acid-fast bacteria other than *Bacillus tuberculosis* give a lesion which in severity and time relation develops much more like the lesion caused by the dead tubercle bacilli than by the living. The exudation is small, the vascularization commences early, and the vessels cease to functionate after a short time. The lesion usually resolves, leaving only a small scar. We have used in this connection the bacillus of timothy, the butter bacillus of Grassberger, Korn's grass bacillus D., the bacilli of frog and fish tuberculosis, an old isolation of the bacillus of avian tuberculosis, and the chromogenic *Bacillus lepræ* of Duval. The last of these cultures has sometimes shown a more progressive and persistent lesion than the others. The difference between *Staphylococcus aureus* and *Bacillus typhosus* on the one hand, and *Bacillus tuberculosis* and the acid-

fast bacteria on the other, in capacity to stimulate the formation of vascular tissue in the cornea is striking. It is, however, a purely quantitative difference or one which is not manifest under more general conditions. This is shown when *Bacillus typhosus* or *Staphylococcus aureus* are inoculated, avoiding the cornea, into the anterior chamber through the sclera. As a consequence of the anterior chamber infection a vascular, circular pannus quickly forms on the cornea.

As described, the corneal lesion due to the inoculation with the tubercle bacillus proceeds in an orderly fashion through certain clearly defined stages and presents in some degree an opportunity for an analysis of the processes involved. Certain experiments were now undertaken to determine whether or not the course of the lesion could be influenced by the local and general administration of various substances. As we hope to develop this phase of the subject in greater detail in the future we shall now merely present our results in outline in so far as they show that the lesion can be modified.

Iodin, Locally Applied as Lugol's Solution.—If the conjunctival sac be washed daily with Lugol's solution the early development of the lesion is in no way influenced. In many instances the pannus when well formed thickens to an unusual degree. The secondary thinning out of the vascular tissue near the corneoscleral margin does not take place. In order to attain this effect the concentration of the iodin in the solution and the duration of the exposure must be sufficient to produce a distinct inflammation when applied to the healthy control eye. The effect may be in large measure an additive one. We have never seen the pannus invade the central exudate under the influence of iodin.

Tuberculin.—Koch's old tuberculin given intravenously in large or small doses or instilled into the eye is without effect on the early development of the lesion. We have not tried its effect later when panophthalmia exists, and our results in no way contradict those of previous observers who have made out a favorable influence from the treatment of experimental and clinical tuberculosis of the eye with this preparation.

Calcium Lactate.—Calcium lactate administered intravenously or subcutaneously in doses sufficient to intoxicate the animal severely is able to inhibit the corneal process in considerable degree. The central exudation perhaps increases more slowly than usual, although this is not perfectly clear. The vascularization of the cornea is somewhat delayed in point of time and proceeds more slowly and much less vigorously than usual.

Benzol.—The subcutaneous administration of benzol in olive oil to the point of severe intoxication decreases the amount of exudation to the inoculation point and causes the vascularization of the cornea to develop slowly and feebly. This experiment like that with calcium lactate cannot be carried to a satisfactory conclusion because it is necessary to give the substance in doses certainly although slowly fatal in order to get demonstrable results. The control blood counts have shown that only in those cases in which the leucocytes were reduced very low has there been an appreciable influence on the development of the lesion.

Intercurrent Disease.—In several instances we have inoculated recently purchased, supposedly normal animals which in the first few days developed an acute illness followed by a period of extreme emaciation. In these animals the development of the corneal lesion was also slow and without vigor. The exudation at the inoculation point was inhibited and until this was well developed there was no vascularization of the cornea.

DISCUSSION.

The foregoing experiments taken in a general sense can hardly be said to differ radically from certain of those previously performed by others. Moreover, when we consider them in detail and attempt to formulate their meaning it is often difficult to be sure that what we have learned is not already fairly well understood by those closely familiar with the pathology of tuberculosis. In spite of a degree of uncertainty as to the final value of such considerations, it is our purpose to state in our own language the point of view at which we have arrived by way of this work and to indicate some of the problems which are defined by it.

Tuberculosis is classed by systematic pathologists as an infectious

granuloma. The justice of this classification is emphasized by these experiments. The corneal lesion consists of a mass of granulation tissue the various elements of which are present in variable proportions at different stages in the development of the disease. The experiments recorded in which the action of *Bacillus typhosus* or *Staphylococcus aureus* is contrasted with that of the tubercle bacillus on the cornea indicate that the latter much more readily gives rise to the formation of blood vessels. This power of stimulating blood vessel formation is also possessed by other acid-fast bacilli. As shown by the results of anterior chamber inoculation, however, the difference is one of quantity rather than of quality. *Bacillus typhosus* and the pyogenic cocci under proper conditions may likewise stimulate blood vessel formation. As a large exudative lesion produced on the cornea with *Bacillus typhosus* may fail to give rise to the new formation of capillaries, while even a minimal lesion with dead tubercle bacilli or with non-virulent acid-fast organisms of other kinds leads to their rapid formation, we are probably justified in believing that the difference is a large one and perhaps of vital importance in the development of the disease. The corneal lesion develops in an orderly way. The irritation due to the injection is recovered from, is followed by a latent or incubation period, and this in turn is followed by the development of a local exudation. Not until the exudate has become definite does the formation of blood vessels begin. In experiments such as those with benzol and calcium lactate in which the primary exudation is limited or suppressed, the blood vessels fail to develop or develop very feebly. Presumably if it were possible to prevent the exudation entirely, the vessels would never develop. Expressed in terms of the prevention of the disease, these results are of little significance. While it is possible to say in these terms that the development of a tuberculous lesion has been definitely delayed by these agencies, yet it has been by methods that are so severe in their general effects that even the experiments can hardly be continued to a satisfactory conclusion.

From another point of view, however, the matter has interest. In the well developed corneal lesion the tubercle bacillus is present in enormous numbers. Several of these inhibitive experiments have lasted from two to three weeks, and in this time the cultures we

have used on culture medium attain a large growth. If in this time there was a great growth of the organism in the transparent cornea it might be expected to become visible; or if the bacteria did not become visible as a colony, the cornea should develop a local defect. As a matter of fact no visible change occurs in the absence of exudation and it seems to be a fair conclusion that we are dealing with a true condition of latency, one in which the bacillus grows little if at all, and in which the tissues are practically unchanged. Stated in another way, the conclusion is indicated that there is nothing in the fixed tissues of the cornea to provide for food for the bacillus and little or nothing about the living tubercle bacillus which does injury to these tissues. With the coming of the cellular exudate the case is entirely different. The products of cellular degeneration furnish food for the bacillus and the fixed tissues are attacked either by the products of the growth of the bacillus in this pabulum or by the products of cellular activity or cellular degeneration. From this heterogeneous combination also, substances are set free which give a stimulus for blood vessel formation and doubtless for the proliferation of other fixed tissue cells.

If this reasoning is in any measure correct, and we believe that it is largely so, we are forced to agree with those who have held that tuberculosis is primarily an exudative inflammation and secondarily proliferative in its character. More than that, we are led to the belief that the cellular exudate, protective though it may be in purpose, is defective and is destructive in its actual influence. Even the phagocytic activity of the cells of such an exudate may probably serve to distribute living bacilli and thus spread the disease to fresh areas.

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THE EXCRETION OF NITROGEN IN FEVER.*

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That there is no qualitative difference between the metabolism of fever and normal metabolism, and no ground for believing that proteins are broken down in infective fevers by the action of toxins upon the protoplasm of the cell, are the conclusions to which Krehl and Grafe (1) have come as the result of work recently done in the laboratories of the Heidelberg clinic.

The evidence adduced by Grafe (2) upon the meaning of the increased nitrogen excretion in fever is partly derived from measurements of the respiratory exchange in fever patients on a fluid diet yielding about 1,200 calories and containing about 5 to 6 gm. of nitrogen, and calculation, from the nitrogen output, of the ratio of the energy derived from protein oxidation to the total amount of energy liberated. This ratio he finds to be similar to that observed in starvation. Partly too (3), from the fact that, if a sufficiently liberal estimate of the energy needs of such patients be made, about 50 calories per kilo, and this amount be supplied in the food, mostly in the form of carbohydrate, nitrogenous equilibrium may be maintained in fever, in most cases when less than 1.4 gm. of protein per kilo of body-weight are given. This amounts, he argues, to proof that the loss of nitrogen often observed in fever is due to the increased evolution of energy in fever and the diminished intake of food. He claims support for this view also from the results obtained by Shaffer and Coleman (4), who prevented loss of nitrogen in typhoid fever by administering very large quantities of carbohydrate. More recently Pfannmüller (5) has succeeded in reducing the output of nitrogen in fever as much as 40 per cent. by adding to an otherwise constant diet 500 gm. of sugar, whereas in patients on this diet whose temperature was normal the addition of this amount of sugar reduced the nitrogen output only about 10 per cent., showing that the largest part of the loss of nitrogen in fever is due to the deficiency of carbohydrate.

It seems clearly established at any rate from all these results that when a liberal supply of carbohydrate can be given it may reduce the destruction of tissue protein in fever. But this result does not necessarily imply that the destruction of protein that would otherwise take place is due merely to a deficiency of calories in the food;

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the presence of large quantities of carbohydrate may affect the course of chemical processes in the body in other ways than merely by furnishing material that is easily oxidized. It is not altogether in harmony with what we know that it should be necessary to supply 50 calories per kilo to a patient in bed even if he is in fever. There is a fair consensus of evidence that the respiratory exchange is increased in fever often 20 or possibly 30 per cent., seldom more than that, and also that 30 calories per kilo is a sufficient supply for a patient lying in bed; if 30 per cent. be added to meet the calls due to the fever, 40 calories per kilo should theoretically suffice in most cases of fever. In Shaffer and Coleman's experiments, it was necessary so to drench the patients with carbohydrate that the calories supplied amounted to 80 or even 100 per kilo.

In some ways the most valuable study of metabolism in fever that has yet been carried out is that of Likhatcheff and Avroroff (6). A woman suffering from malaria was examined in the calorimeter on days on which attacks occurred, and also when her temperature remained normal, but conditions were otherwise similar. With a normal temperature her heat production was 32 calories per kilo per day. On a day when her temperature rose about 3° C. to 39.3° heat production rose to a little more than 35 calories per kilo.

It is difficult to see why, in order to prevent so small an increased evolution of energy as this resulting in the destruction of proteins in the body, it may be necessary to supply energy at a rate two or even three times as great as this, most of it in the form of carbohydrate, if the protein is attacked merely because there is not carbohydrate available.[1] The experiments of Kocher (7) recently reported showed that a diet of 70 calories per kilo containing only 2 gm. of nitrogen, which in patients whose temperature was normal gave an output of nitrogen amounting only to 3 gm., resulted in the loss of much larger amounts of nitrogen, up to more than 20 gm., in the case of patients in fever.

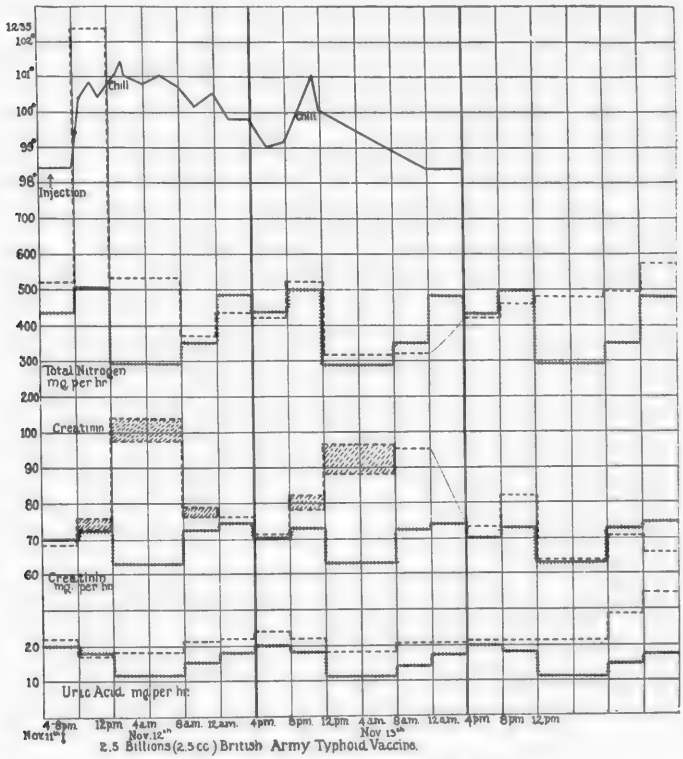
[1] It is true that this may not be a fair way of looking at the case in point. The energy output for the day on which the fever occurred amounted, when reckoned for the twenty-four hours, to only 10 per cent. more than on a normal day. But a valuable feature of the experiment is that the effect is followed not merely by the coarse method of twenty-four-hour periods, as is the case with most work on fever. A fine adjustment to two-hour periods was made use of, and it becomes apparent that between the hours of 3 and 6 A. M. the rate of heat production rose to 110 calories per hour when at these hours with a normal temperature it would have been only about 60 calories per hour. Such a sudden short burst of racing heat production might possibly be supposed to overstrain the immediately available supply of carbohydrate, and temporarily necessitate recourse to protein material; only it must be remembered that still greater departures from the usual rate of heat production, in muscular exertions, can be borne, as is well known, without any such result.

The principal difficulty in the study of metabolism in fever consists in the establishment of proper controls with which to compare the abnormal change. Controls obtained from the same individual during convalescence are not always necessarily satisfactory, and they are generally the best that can be obtained. The ideal condition would be to have the subject under examination for some days before the onset of the fever and to be able to keep the diet constant all the time. In the case of malaria this might be possible, but hardly in that of any other disease. But something may be done with the febrile reaction induced by the injection of toxins. Two experiments of this kind done on the same subject with typhoid vaccine were reported by Leathes in 1907 (8), and we have done two more on two other subjects with typhoid vaccine and four on four different subjects with tuberculin following essentially the method employed by him; we have also examined a patient suffering from malaria.

The procedure adopted consists in establishing the normal rates at which total nitrogen, creatinin, and uric acid are excreted by the subject at different periods of the day, by collecting the urine at fixed hours several times daily for several days on a constant diet. After a week the average output for each period of the day is calculated and plotted on squared paper representing the normal rate of excretion for that individual on that diet. The injection is timed so as to bring on the reaction in the evening and the maximum temperature in the night so that the intake of food is not interfered with at all and the disturbance of nutrition that it is otherwise so hard to allow for is entirely eliminated. On the next day the temperature has generally fallen; no meals are missed and the diet remains the same. The rates at which total nitrogen, creatinin, and uric acid are excreted during each period following the injection are then plotted on the same paper with the normal average rates previously obtained, and any departure from the normal becomes at once apparent. In this way the changes effected can be followed more minutely, and pronounced departures from the normal course can be detected, which being of short duration might otherwise be lost sight of in averaging up the whole output for twenty-four hours.

The results obtained are presented in the form of curves in text-figures 1 to 8. Text-figure 3 gives for comparison the results of

one of the experiments referred to above and previously reported by Leathes. The abscissæ represent time, divided into periods corresponding to the times at which the samples of urine for analysis were collected, generally every four hours. The ordinates give



TEXT-FIG. 1.[2] Case 1. Typhoid vaccine.

the hourly rate of excretion of either total nitrogen, creatinin, or uric acid, as indicated on the scale at the left hand side. In each case the dotted interrupted line shows the hourly rates for each period

[2] In the text-figures, the black lines represent the normal rate of excretion in mg. per hour of uric acid, creatinin, and total nitrogen, being the average of several days under fixed conditions, divided in four-hour periods. The dotted lines represent the rate in mg. per hour following the injection.

following the injection; the average normal hourly rates calculated from six to eight days on the same diet preceding the injection are shown as continuous lines (in text-figure 1 these lines have cross markings), and being normal averages repeat themselves every twenty-four hours. The charts for convenience have black vertical lines running through them dividing them into periods of twenty-four hours. The temperature curve is given so as to show the relation of the changes in the rates of excretion to the changes in body temperature.

The diet was in most cases milk and bread and butter; in some cases it included eggs and cheese or fruit. No meat or meat broths were included in any case. In all cases as far as possible the composition and amount of food and liquid taken were the same on each day of any given experiment and they were taken at the same hours throughout that experiment.

Creatinin was estimated by Folin's colorimetric method with bichromate solution for standard; uric acid by his modification of Hopkins' method; total nitrogen by Kjeldahl's method. Creatin was looked for in all cases except the experiment charted in text-figure 3; when found the amount is indicated by a shaded area in the creatinin curve.

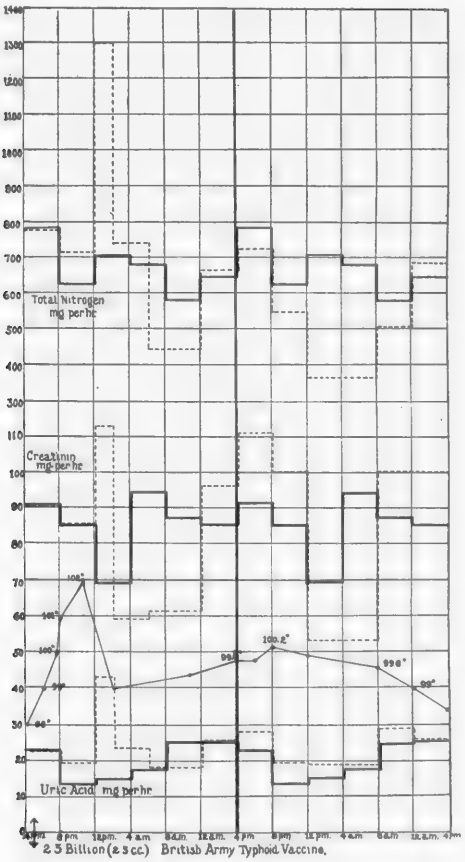
The time at which the injection was given is marked by an arrow on the chart.

If now the first three curves which give the results of experiments with typhoid vaccine are consulted, it will be observed that they agree in showing a sharp rise in the excretion rates synchronizing closely with the rise of temperature, and that they agree also in showing a greater change in the excretion of uric acid than of either creatinin or total nitrogen. When the reaction occurs the excretion of uric acid in case 1 is increased more than 60 per cent. for eight hours, and for more than twenty-four hours after that remains uniformly increased. In case 2 it is nearly trebled for two hours, and in case 3 it is more than trebled for eight hours and remains above the normal for several hours after that.

In table I the quantities excreted in twenty-four-hour periods after the injection, as compared with the normal twenty-four-hour average, are given. It illustrates how a marked reaction may be

missed if the whole of the urine for twenty-four hours is analyzed together, most notably in case 2.

In case 1 the uric acid in the blood was estimated on three days at the same hour by Folin's colorimetric method. On two days before



TEXT-FIG. 2. Case 2. Typhoid vaccine.

the injection the amount found in double estimations was 0.82 and 0.81 of a milligram per 100 cubic centimeters on the first, and 0.80 and 0.77 on the second. On the day when the injection was given,

TABLE I.

Typhoid Vaccine.

Total Excretion in Periods of Twenty-Four Hours.

	Total nitrogen.		Uric acid.		Creatinin.	
	Gm.	± per cent.	Gm.	± per cent.	Gm.	± per cent.
Case 1. N. C. S.						
Normal average	8.9		0.36		1.64[3]	
1st 24 hrs. after injection	14.4	+61	0.48	+33	2.02[4]	+23
2d 24 hrs. after injection	9.2	+3	0.48	+33	2.05[4]	+25
3d 24 hrs. after injection	11.8	+33	0.60	+67	1.68[3]	+2
Case 2. K. M. B. S.						
Normal average	16.1		0.46		2.10[3]	
1st 24 hrs. after injection	16.8	+4	0.55	+20	1.90[3]	−10
2d 24 hrs. after injection	13.0	−20	0.56	+21	2.10[3]	
Case 3. J. L.						
Normal average	7.6		0.44		2.07[5]	
1st 24 hrs. after injection	9.8	+28	0.71	+61	2.24[5]	+8

and seven and a half hours after it, the amount found was 1.75 milligrams per 100 cubic centimeters. In the urine that had been excreted during those hours the uric acid corresponded, as may be seen in text-figure 1, to the amount excreted at that time on normal days; but immediately after that the rate of excretion was increased and remained so for more than forty-eight hours. This result shows that the increased excretion cannot be accounted for merely by increased renal activity, but corresponds to a really increased production.

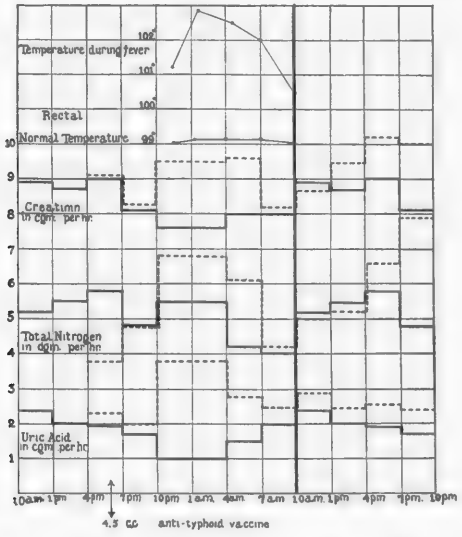
We find it difficult to reconcile entirely such results as these with the conclusions to which Krehl and Grafe have come with regard to the interpretation of the increased nitrogen excretion in fever. It is difficult to see how these marked reactions can be put down to a sudden deficiency of carbohydrate; the subjects were in good condition, well nourished, strong, healthy, and capable at any moment like other healthy subjects of doubling or trebling their ordinary rate of discharge of energy by muscular exertions without showing any such change in the nitrogen excretion. Moreover, it appears to us that there is evidence of a qualitative change in metabolism during

[3] No creatin found.

[4] Including creatin.

[5] Creatin not estimated.

a febrile reaction to be detected also in the way in which the uric acid is more affected than other forms of nitrogenous excreta. The reaction of the body to the typhoid toxin seems to consist not in a rise of temperature due, so far as it is due to increased heat production, to a general acceleration of metabolism qualitatively the same as that which was proceeding before the reaction was excited, but to chemical processes of a special kind. Whether these processes are merely certain special factors in normal metabolism that are necessarily exaggerated in the heightened activities, nervous or muscular

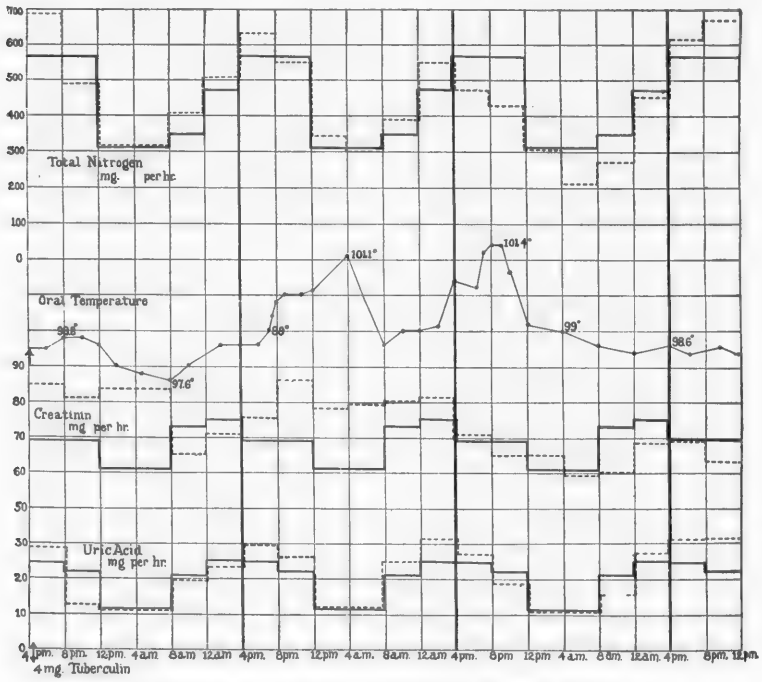


TEXT-FIG. 3. Case 3. British Army typhoid vaccine.

or of whatever nature they may be, that are involved in the reaction, or whether they are toxogenic and due to the damage done to cell protoplasm by the toxin, it is not possible from these data alone to decide. Possibly a comparison of the results of the injection of typhoid vaccine with the changes brought about by tuberculin, to which we now turn, may help to decide this question.

The results of tuberculin injections when studied in this way in four subjects, two of them normal, one slightly, the fourth

severely infected, are given in text-figures 4 to 7 and table II. It is clear that the reaction to this toxin is slower than the one already dealt with. Except in the case of the subject who was severely infected (case 7), about twenty-four hours elapse before the temperature rises, and the rise of temperature when it occurs tends to be less. The abrupt change in the rates at which nitrogenous ex-



TEXT-FIG. 4. Case 4. Tuberculin. Normal subject.

creta are turned out, that accompanies the prompt rise of temperature after typhoid vaccination, is missed in cases 4, 5, and 6, which reacted slowly, and is noticeable in case 7, which reacted more rapidly to tuberculin, only after the first injection. But in that instance the rise of temperature was little more than 1° F., and similar temperature reactions to a second and third dose, though these doses were larger, were accompanied by less definite changes in the excretion.

TABLE II.

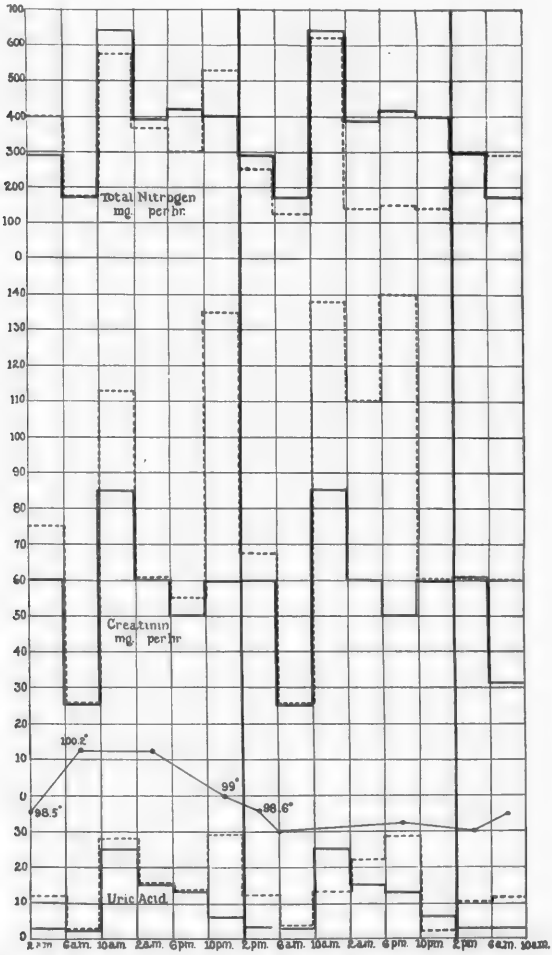
Tuberculin.

Total Excretion in Periods of Twenty-Four Hours.

	Total nitrogen.		Uric acid.		Creatinin.	
	Gm.	± per cent.	Gm.	± per cent.	Gm.	± per cent.
Case 4. N. C. S. (No tuberculosis)						
Normal average	10.2		0.45		1.6	
1st 24 hrs. after injection	10.4	+ 2	0.41		1.8	+13
2d 24 hrs. after injection	10.9	+ 7	0.52	+15	1.9	+19
3d 24 hrs. after injection	8.3	−19	0.41	− 9	1.4	−13
Case 5. C. L. (No tuberculosis)						
Normal average	8.9		0.26		1.3	
1st 24 hrs. after injection	12.6	+42	0.46	+77	1.9	+46
2d 24 hrs. after injection	8.8		0.26		1.8	+40
Case 6. W. P. (Slight tuberculosis)						
Normal average	9.8		0.22		0.84	
1st 24 hrs. after injection	10.5	+ 7	0.30	+36	1.10	+31
2d 24 hrs. after injection	9.9		0.34	+55	0.94	+12
3d 24 hrs. after injection	10.7	+ 9	0.24	+ 9	0.85	
Case 7. R. K. (Marked tuberculosis)						
Normal average	12.7		0.46		1.22	
1st 24 hrs. after injection	13.4	+ 5	0.50	+ 9	1.24	
2d 24 hrs. after injection	12.6		0.46		12.5	

The change in the output for twenty-four-hour periods shown in table II is considerable in cases 4, 5, and 6 in regard to creatinin, in cases 5 and 6, but less so in case 4 in regard to uric acid, in case 5 and less so in case 6, in regard to total nitrogen. Case 4 showing the reaction to tuberculin in the same subject as case 1 to typhoid vaccine is particularly instructive, especially as the temperature rose to the same level in both experiments, and the marked effect on the output of uric acid in the reaction of this subject to typhoid vaccine is missed in the reaction to tuberculin. The reaction to typhoid was accompanied by definite rigors, that to tuberculin was not. There does not therefore appear to be the same uniformity in the reaction of different subjects to tuberculin as in the reaction to typhoid vaccine. And the most striking feature in the reaction to typhoid vaccine, the sudden change in output of all the forms of nitrogenous excreta in-

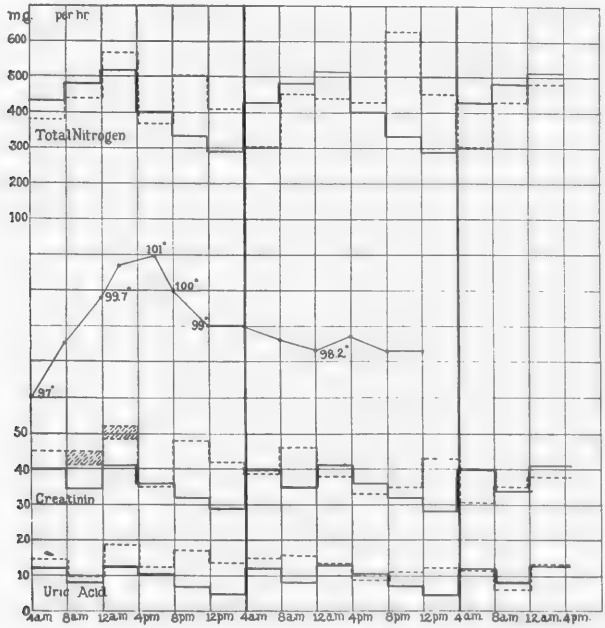
vestigated, accompanying the rapid rise of temperature, is not to be observed with the slower reaction to tuberculin. But in the reac-


Total Nitrogen mg. per hr.
Creatinin mg. per hr.
Uric Acid
100.2°
98.5°
99°
98.6°
2 p.m. 6 a.m. 10 a.m. 2 a.m. 6 p.m. 10 p.m. 2 p.m. 6 a.m. 10 a.m. 2 a.m. 6 p.m. 10 p.m. 2 p.m. 6 a.m. 10 a.m.

TEXT-FIG. 5. Case 5. Tuberculin, 1 mg. eighteen hours before. Normal subject.

tion to tuberculin as in that to typhoid vaccine the changes that occur in the output of nitrogenous substances are such as to make it diffi-

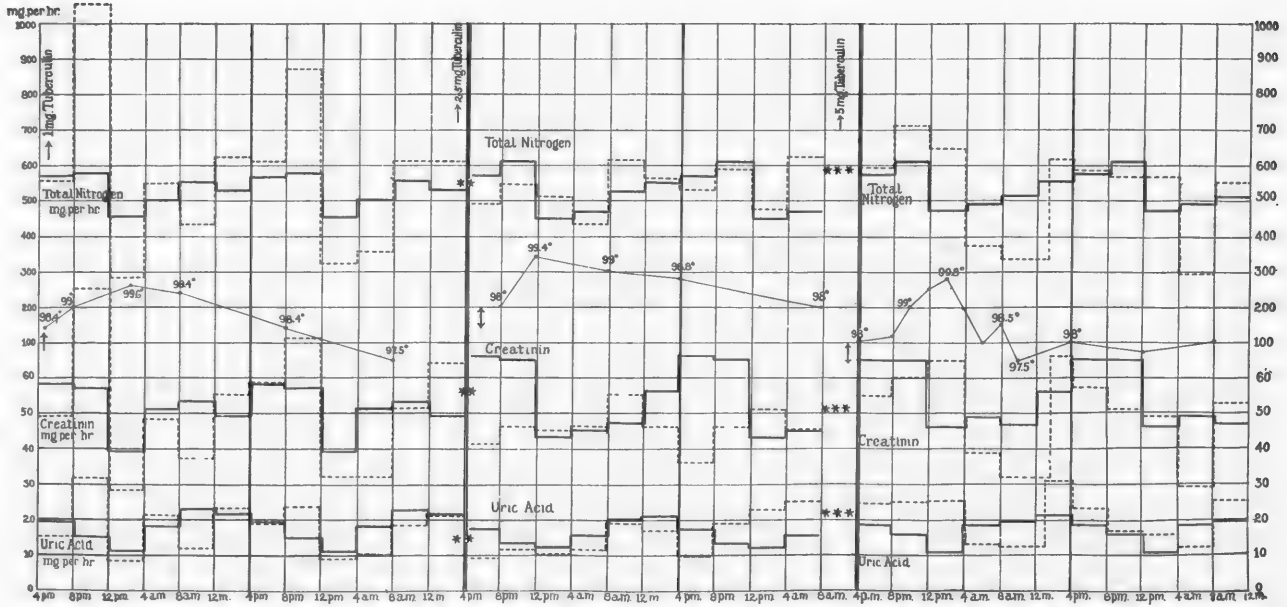
cult to concur in the conclusion that there is no qualitative difference between the metabolism in fever and normal metabolism. Whether the other fact which these experiments bring out, namely that the effects of the two toxins are different from each other in certain respects, should be interpreted as showing that the increased output is not due to increased activities involved in the process of raising



TEXT-FIG. 6. Case 6. Tuberculin, 2 mg. sixteen hours before. Subject suffering from slight tuberculosis.

the body temperature, and involved therefore alike whichever toxin was used, but is due rather to the two poisons attacking the cell protoplasm in ways that are different, though both of them cause a toxogenic nitrogen excretion, this is a question which the data are not yet sufficient to determine.

Finally there remains the case of malaria. In this instance, however, it was impossible to get normal control figures in the way



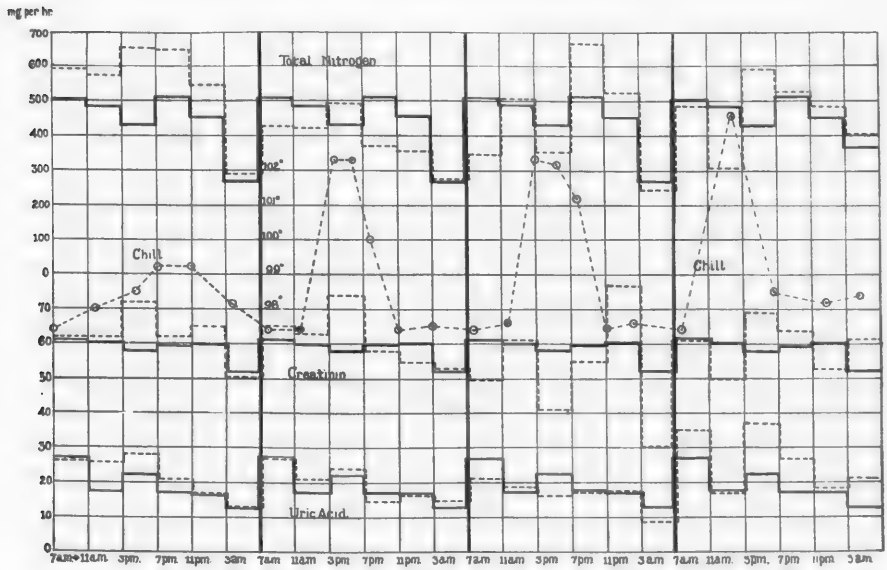
TEXT-FIG. 7. Case 7. Tuberculin, 3 doses as indicated in the text-figure, in a subject suffering from well marked tuberculosis.

TABLE III.

Malaria (Double Tertian).

Case 8. V. K.	Total nitrogen.		Uric acid.		Creatinin.		Maximum temperature F.	Remarks.
	Gm.	± per cent.	Gm.	± per cent.	Gm.	± per cent.		
1st dy.....	13.2	+26	0.52	+16	1.49	+ 6	99.2°	Rigor.
2d dy.....	9.4	−10	0.48	+ 7	1.47	+ 5	102.3°	
3d dy.....	10.5	± 0	0.39	−13	1.26	−10	102.3°	
4th dy.....	11.2	+ 7	0.62	+38	1.43	+ 2	103.6°	Rigor.
5th dy.....	10.5		0.45		1.40		98.2°	Quinin.

The percentage variations are calculated with reference to the 5th day as standard, when quinin was given and there was no rise of temperature.



TEXT-FIG. 8. Case 8. Malaria.

in which this was done with the other cases. The patient had had several days of fever before he came under observation. Analyses were carried out on four days on which there was fever, on two of them preceded by rigors, and on a fifth day when administration of quinin prevented both rigor and fever. The figures obtained on the fifth day are charted as the continuous line and are given for com-

parison with those for each twenty-four-hour period on which fever occurred. As far as the available data go they show higher figures for uric acid and total nitrogen on the days on which there was rigor than on any of the others, and the fact that the effect of the reaction on the excretion of nitrogenous substances is different from that observed with the typhoid vaccine and also from that after tuberculin is perhaps in favor of the view that different pyretogenic toxins affect the excretion of nitrogen in different ways because the damage done to the cell protoplasm is different. But the lack of satisfactory controls makes the figures obtained from this case less significant (table III).

CONCLUSIONS.

The rise of temperature following typhoid vaccination is accompanied by a marked increase in the output of total nitrogen, creatinin, and especially of uric acid.

The reaction to tuberculin is slower, but is also accompanied by an increased output of nitrogenous substances, that is, however, less uniform and generally less pronounced.

In a case of malaria there was a tendency for the rise of temperature to be accompanied by an increased output of total nitrogen and less uniformly of creatinin; on two days on which the fever was preceded by rigor the output of uric acid was increased.

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LIVER FUNCTION AS INFLUENCED BY THE DUCTLESS GLANDS.*

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In recent publications[1,2] we have shown that valuable information concerning liver function and liver disease can be obtained by the intravenous injection of a solution of phenoltetrachlorphthalein. The drug is excreted through the bile by the activity of the hepatic epithelium and may be extracted from the feces and estimated with considerable accuracy. The phthalein excretion of normal dogs is quite constant, as is shown by the tables given below. The normal base line curve of phthalein excretion for an individual dog under uniform conditions is still more constant.

We have reported elsewhere[3] experiments that indicate that injury of the liver by means of specific poisons such as chloroform, phosphorus, and hydrazin causes a fall in the phthalein excreted in the feces. The fall in the phthalein curve is proportional to the amount of liver injury and in acute fatal poisoning may reach zero. Given a definite liver injury it is found that a definite amount of the phenoltetrachlorphthalein will appear in the urine, which is free from the drug under normal conditions. Moreover, injury of the liver by actual cautery will cause a definite drop in the phthalein curve, depending upon the amount of injury and inflammatory reaction.

Under various conditions in which the functional capacity of the liver cell is interfered with, the output of phthalein falls below

* Received for publication, June 23, 1914.

[1] Whipple, G. H., Mason, V. R., and Peightal, T. C., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 207.

[2] Whipple, G. H., Peightal, T. C., and Clark, A. H., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 343.

[3] Whipple, Peightal, and Clark, *loc. cit.*

normal, depending upon the extent of the derangement. For example, in passive congestion of the liver, either experimental or clinical, there will be periods of decreased phthalein output corresponding to the intensity of the stasis or breaks in cardiac activity. The Eck fistula (dog 12–2) provides evidence in the same direction. When the portal blood is shunted around the liver by this means we may not at first get a decrease in liver function, but with the development of liver atrophy and fatty degeneration, a distinct and sometimes a marked depression in the excretion curve will be noted.

It is clear that injury or any definite functional derangement of the liver is associated with a definite change in its excretion of phenoltetrachlorphthalein. Passive congestion and Eck fistula are of especial importance in connection with the experiments given below for they show that a change in nourishment of the liver cell leading to atrophy and perhaps fatty degeneration can be recognized by this functional test.

It is important to recall that shortly after repair of an injury by chloroform or phosphorus the liver may give evidence of hyperactivity and excrete more phthalein than under normal conditions. The same may occur after a small dose of a liver poison, which we may assume acts as an irritant to the liver cells. Young dogs and pups will, as a rule, show a slightly higher excretion than adult or old dogs.

All the evidence suggests strongly that a decrease in functional capacity of the liver is paralleled by a lowered phthalein output. May we not assume that a decrease in the phthalein output means a decrease in the functional capacity of the liver? We have studied the liver function in the pathological states brought about by removal of the various ductless glands. The liver function, as indicated by the phthalein excretion is definitely impaired when the body is suffering from pancreatic or adrenal insufficiency. The liver may show no histological evidence of this change in function, but a constant fall in the phthalein curve occurs which may return to normal if, in the case of partial adrenal extirpation, the remaining fragment undergoes an hypertrophy sufficient for the needs of the body.

After complete pancreatic extirpation there is a steady fall in

phthalein excretion which may fall as low as one third or one fifth normal. This fact speaks strongly for the idea that pancreatic activity is essential to proper liver function, which is the case with the adrenals; and it contradicts the idea that the pancreas tends to inhibit liver activity, while suggesting that the pancreas may be looked upon as having an accelerating influence on the liver. This may have some relation to recently proposed theories of diabetes.

Hypophysis extirpation may cause fluctuations in the excretion curve,—an initial fall and secondary rise. A definite fall occurs shortly before death and is noted before the appearance of subnormal temperatures which may supervene a day or so before death.

Thyroid extirpation causes no change in the excretion curve unless the parathyroids are interfered with. Perhaps in experiments of longer duration we may be able to demonstrate some change in liver function.

Parathyroid extirpation and tetany cause no fall in the phthalein excretion curve, but rather a slight rise above normal. In the last day of fatal tetany the liver function will be normal or slightly above normal, which is remarkable considering the prostration that is present at this time. Also given a falling curve due to pancreas extirpation one can produce a rise in output by parathyroidectomy and tetany, a fact which speaks in favor of the supposition that the parathyroid glands exert directly or indirectly inhibitory action upon the liver.

When we recall the amount of injury that must be done to the liver to cause a definite fall in the phthalein excretion curve, it is obvious what marked influence the adrenals and especially the pancreas must exert upon the liver. It is more than possible that this derangement in liver function may be an important factor in the symptom complex of ductless gland insufficiency.

METHOD.

The method employed has been described in detail in an earlier publication,[4] but some points will be reviewed here. The tetrachlorphthalein solution used for the injections was made up in 100 to 200 c.c. lots in 2.5 per cent. strength, as it was found that such a solution does not precipitate within a couple of weeks if kept in a dark place. The method of preparation has been previously

[4] Whipple, Peightal, and Clark, *loc. cit.*

described.[5] This solution for injection was standardized with great care against a known solution containing 10 mg. of tetrachlorphthalein to the liter. This insures the injection of 100 or 200 mg. at each injection and makes for constant excretion. The experiments were done in groups using from three to six dogs of which one normal animal served as a control. The solution was injected using a glass syringe and needle in the jugular vein. Injections were usually made about noon and on the following morning a purge of magnesium sulphate insured complete evacuation of the phthalein-containing feces. A second collection must be made to insure complete evacuation of the drug, but will usually be found to contain only a faint trace, provided the purgation has been satisfactory. It is important to clean the feces from the metabolism cages with great care, using a wash bottle and spatula. It is essential to add about 5 c.c. of alkali to the liter wash bottle, as this shows at once any traces of phthalein which otherwise might be overlooked and helps in the solution of the drug.

The method of extracting the phthalein from the feces may be outlined briefly as follows: The collected feces are diluted to one or two liters including 10 c.c. of strong sodium hydroxide (40 per cent.) and shaken in a machine until a uniform fluid is obtained. One tenth of this mixture is diluted to 500 c.c. with water and 4 to 5 c.c. of strong sodium hydroxide. Of this 500 c.c. solution, 100 c.c. are taken for precipitation with the calcium solution,—calcium chloride 20 per cent. To this 100 c.c. are added 5 to 10 c.c. of the calcium solution, followed by 5 c.c. of strong sodium hydrate and water up to 200 c.c. This effects a final dilution of one tenth of the feces to one liter, including the alkali and calcium solutions. This solution may be filtered at once and read against a standard phthalein solution containing 10 mg. per liter. The readings will be directly in per cent. if 100 mg. have been injected.

It will be noted that the phthalein excretion in normal dogs is constantly higher than in our last report. This is due to a more accurate method for standardizing the injection solution which in these experiments contained 100 mg. in each 5 c.c. In our earlier experiments the solutions were of constant strength, but the amount of phthalein was overestimated giving a lower excretion in the feces. All these dogs have been injected with the correctly standardized phthalein so that no fluctuations in the curve are attributable to this factor except some of the earlier observations in the first experiment (Eck fistula, dog 12–2) which were made during the year 1912–1913.

EXPERIMENTAL OBSERVATIONS.

In all operations the dogs were given morphia before operation and ether anesthesia during the operation, which in no instance lasted more than one hour. The usual aseptic surgical technique was employed and great care exercised in the postoperative treatment to insure a rapid return to normal if possible.

[5] Rowntree, L. G., Hurwitz, S. H., and Bloomfield, A. L., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 327.

ECK FISTULA OF EIGHTEEN MONTHS' DURATION.

Dog 12–2.—Black and tan mongrel, female.

Feb. 1, 1913. Ether anesthesia. Eck fistula produced as usual with ligature of portal vein at hilum of liver.

Feb. 3.. Dog in good condition; weight 13½ pounds. The dog presented the usual picture seen in successful Eck fistula experiments, having occasional digestive upsets, but in general maintaining normal weight with gradual increase in strength and general activity. Phthalein excretion is shown in the accompanying table, and in part has been recorded elsewhere in an earlier report. The condition has been practically unchanged during the year succeeding the last observation. There were slight fluctuations in the weight curve, but the condition during this time has been uniformly excellent.

June 10, 1914, 12 M. Dog in excellent condition. Weight 16 pounds. Phthalein 0.1 gm. intravenously.

June 11. Urine contains a good deal of phthalein as usual. No feces.

June 12. Abundant fluid feces. Phthalein excretion 45 per cent. 4 P.M. Ether anesthesia and bleeding from femoral. Weight 15½ pounds.

Autopsy.—Performed at once. Thorax, heart, lungs, and spleen are all quite normal. Pancreas, kidneys, adrenals, thyroid, and parathyroid all normal. Stomach and intestinal tract normal. Eck fistula shows clean margins and an opening about 3 by 4 mm. The portal vein is completely obliterated at the site of the ligature, and there are no collaterals above this ligature. There are large collaterals in the neighborhood of the kidney, forming easy collateral circulation between the portal and lumbar veins. Liver after bleeding and removal of gall bladder weighs 189 gm. This is to be compared with the normal dog 13–45, weight 15¼ pounds, killed at the same time under the same conditions. Normal liver without gall bladder weighed 232 gm. The Eck fistula liver shows regular lobulation and definite evidence of fatty change. The bile passages are everywhere normal.

Microscopical Examination.—Liver shows a remarkable grade of fatty degeneration involving about all of the central two thirds of each lobule. The

DOG 12–2.

Eck Fistula of Eighteen Months' Duration.

Date.	Phthalein in gm.	Phthalein excreted, per cent.		Weight in pounds.	Remarks.
		Feces.	Urine.		
Feb. 1, 1913...	—	—	—	13.5	Eck fistula produced.
Apr. 1.....	0.10	17	++	15.0	
Apr. 8.....	0.10	20	0.25	16.0	
Apr. 15.....	0.10	30	2.0	15.5	
May 26.....	0.10	36	0.1	17.0	Dog improving.
June 17.....	0.10	40	0.4	17.0	
July 10.....	0.10	40	0.9	16.3	
Oct. 27.....	0.10	34	1.0	15.7	Dog in good condition.
Apr. 27, 1914 ..	0.10	46	0.5	16.0	
June 10.....	0.10	45	++	16.0	
June 12.....	0.10			15.5	Autopsy.

liver cells contain large droplets of neutral fat and the nuclei stain sharply. There are no phagocytes nor any evidence of cell necrosis. The marginal third of each lobule is normal. Bile passages and canaliculi are normal. Other organs negative.

The preceding experiment (dog 12–2, Eck fistula) shows several points of interest. A dog can live in health for a year and a half with an Eck fistula. This dog had a liver smaller than a healthy normal dog of the same weight, and the Eck fistula liver was supplied by about one third the normal amount of blood, yet excreted about two thirds the normal amount of phthalein. This is a simple example of atrophy and slight fatty degeneration due to decreased or changed blood supply. The dog showed no clinical evidence of liver insufficiency and appeared to be in perfect health, yet this physiological test shows a uniformly subnormal output of phthalein. It must be kept in mind that the mere presence of an Eck fistula does not effect a drop in the phthalein curve which may remain normal for some time after the operation, but the drop in the curve follows when the liver atrophy develops. This comes out in experiments previously reported.[6] Another point of interest in this dog and others of similar type is that pregnancy will not develop. The dogs appear normal in all respects and may go in heat frequently and take the male repeatedly, but no pregnancy follows.

After the first few weeks when the animals are given plenty of bones and milk with no meat, these animals may be kept indefinitely on the usual mixed diet of cooked meat and bread cakes. They do not stand confinement in cages as well as normal animals, and exercise is needful. These observations bear on the question of operation in liver disease in man, and it seems that a suitable technique for the operation is all that is required before applying the Eck fistula operation successfully to human cases of cirrhosis in which the symptoms of portal obstruction are the dominant ones.

ADRENAL EXTIRPATION.

Dog 13–56.—Young male pup; weight 14 pounds.
Jan. 23, 1 P.M. Phthalein 0.1 gm. intravenously.
Jan. 24. Abundant feces. Phthalein excretion 68 per cent.
Jan. 27, 11 A.M. Ether anesthesia. Right adrenal removed completely. Left

[6] Whipple, Peightal, and Clark, *loc. cit.*

adrenal cut across, and the lower pole including about one third of the gland parenchyma left *in situ*. The upper two thirds were removed.

Jan. 28. Dog is quite weak, pulse fair, wound dry; weight 12¾ pounds. Phthalein 0.1 gm. intravenously.

Jan. 29. Diarrhea marked. Phthalein excretion 44 per cent.

Feb. 3 and 4. Dog improving and quite lively.

Feb. 7, 3 P.M. Dog appears normal; weight 13¼ pounds. Phthalein 0.1 gm. intravenously.

Feb. 8. Dog has diarrhea without any purgation. Phthalein excretion 52 per cent.

Feb. 10. Diarrhea continues.

Feb. 21, 12 M. Dog in good condition. Weight 13¾ pounds. Phthalein 0.1 gm. intravenously.

Feb. 22. Spontaneous diarrhea. Phthalein excretion 39 per cent.

Feb. 25. Soft feces and fluid stools persisted.

Apr. 8, 1 P.M. Dog in good condition; weight 16 pounds. Phthalein 0.1 gm. intravenously.

Apr. 10. Formed feces. Phthalein excretion 70 per cent.

May 1, 3 P.M. Dog is normal. Operation under ether anesthesia. Left adrenal shows very definite hypertrophy. The fragment is more than double its former size. Fragment cut transversely, the lower half removed and the upper half left intact.

Pancreas.—Both upper and lower arm of gland extirpated, but the parenchyma adherent to the duodenum was left undisturbed. Probably three fifths of pancreatic parenchyma were removed and the blood supply to the remainder left undisturbed.

May 2. Dog in good condition.

May 3, 12 M. Dog appears normal. Wound is dry. Weight 16 pounds. Phthalein 0.1 gm. intravenously.

May 4. No feces. Urine contains no sugar. 5 P.M. Fluid feces. Phthalein excretion 52 per cent.

May 6. Dog vomits occasionally, but has no diarrhea; weight 16¾ pounds. Phthalein 0.1 gm. intravenously.

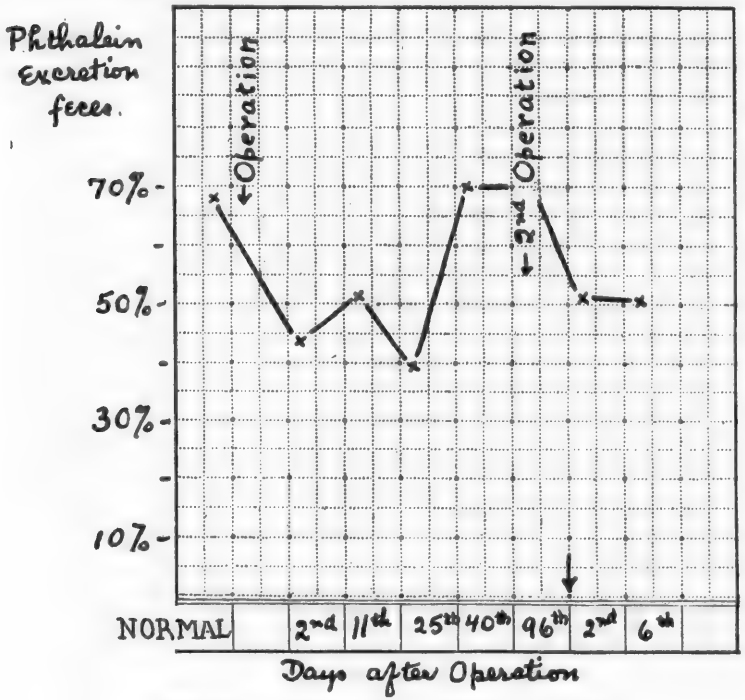
May 7. Abundant fluid feces. Phthalein excretion 51 per cent.

May 17. Dog appears quite sick and suffers from dyspnea. Temperature 35° C.

May 18. Found dead.

Autopsy.—Performed at once. The striking thing is the general anasarca. There is marked subcutaneous edema and accumulation of clear yellow fluid, about 300 c.c. in the peritoneal cavity, and almost the same amount in the thorax. The pericardial cavity is clear. Heart contains no clots. It shows dilatation and hypertrophy of the right side. Lungs show emphysema and small pulmonary thrombi. Spleen, kidneys, and intestinal tract normal. Liver is rather large, and lobules are conspicuous, due to edema. Pancreatic remnant looks normal. There are adhesions about the sites of operation. Adrenal fragment has increased in size to almost that found at the second operation. Cortex shows very marked hypertrophy. The cause of the general anasarca is quite obscure.

Microscopical Examination.—Adrenal gland removed at second operation shows the hypertrophy and increase in fat described by Crowe[7] in a recent publication. The adrenal fragment examined at autopsy shows the same picture together with a certain amount of necrosis, probably dependent upon interference with the blood supply. The parenchyma cells in places look much degenerated. There was undoubtedly a good deal of interference with the adrenal parenchyma and probably a marked insufficiency. The lungs show small patches of organizing pneumonia and induration. Pancreas and kidney are normal. Liver shows central congestion with slight atrophy and fatty degeneration. Some sections show definite central necrosis with thrombosis of adjacent capillaries.



TEXT-FIG. 1. Dog 13–56. Adrenal extirpation.

The preceding experiment (dog 13–56, text-figure 1) shows clearly the depression of liver function depending upon adrenal insufficiency. With hypertrophy of the remaining adrenal fragment

[7] Crowe, S. J., *Bull. Johns Hopkins Hosp.*, 1914, xxv (in press).

the liver function returns to normal, as would be expected. The second operation in which one half of the remaining adrenal fragment was removed was followed by a similar drop in phthalein excretion. At this second operation a portion of the pancreas was removed, but this procedure by itself would have no effect upon liver function. The cause of the anasarca is obscure, but may have been due in part to the changes in the pulmonary circulation causing hypertrophy of the right heart and more or less venous stasis.

The following experiment (dog 13–94) shows that a small adrenal fragment is more efficient in maintaining normal liver function when it is in connection with the sympathetic system by means of its nerves. Furthermore, a simple section of the large nerves going to this upper pole and no interference with the blood supply caused a depression in the phthalein curve when the first operation and removal of three fourths of the gland parenchyma had given no evidence of insufficiency.

ADRENAL EXTIRPATION. NERVE SECTION.

Dog 13–94.—Small fox-terrier, male; weight 13 pounds.

Mar. 20, 12 M. Phthalein 0.1 gm. intravenously.

Mar. 21. Abundant feces. Phthalein excretion 69 per cent.

Mar. 28, 11 A.M. Operation under ether anesthesia. Right adrenal removed completely. Left adrenal cut across leaving the upper two fifths of the gland intact, the lower three fifths being removed.

Mar. 29, 11 A.M. Dog in fair condition. Phthalein 0.1 gm. intravenously.

Mar. 30 and 31. Abundant feces. Total phthalein excretion 71 per cent.

Apr. 8. Dog very active; weight 13 pounds. Phthalein 0.1 gm. intravenously.

Apr. 9. Abundant feces. Phthalein excretion 74 per cent.

Apr. 25, 12 M. Operation under ether anesthesia. Left adrenal exposed with very little bleeding and no interference with the blood supply. The large nerve bundle to the upper pole was isolated and cut. The adrenal was handled very little and only this large nerve disturbed. Duration of operation about thirty minutes, which was about half the time required for the first operation, and there was much less handling of viscera during this second operation.

Apr. 26, 11 A.M. Dog is lively and appears normal in all respects. Weight 13¼ pounds. Phthalein 0.1 gm. intravenously.

Apr. 27 and 28. Abundant feces. Total phthalein excretion 59 per cent.

May 3, 12 M. Phthalein 0.1 gm. intravenously. Weight 13½ pounds.

May 4. Abundant feces. Phthalein excretion 72 per cent.

June 11. Dog normal. Weight 15 pounds. Ether anesthesia and bleeding from femoral.

Autopsy.—Performed at once. Thorax, heart, and lungs are normal. Thymus is large, milky, and soft. Testicles are of adult type. Lymph glands are not particularly enlarged, nor are the lymph follicles conspicuous in the intestine and spleen. The liver is quite normal. Pancreas, kidney, thyroid, and parathyroid are normal. Adrenal fragment has increased considerably in size. On section the cortex and medulla appear to be both involved in this hypertrophy.

Microscopical Examination.—Liver is normal. Thymus and lymphatic tissue show considerable hyperplasia. Adrenal shows the usual hyperplasia, especially of the cortex. Other organs normal.

DOG 13–94.

Adrenal Extirpation (Partial).

Date.	Phthalein in gm.	Phthalein excretion in per cent. in feces.	Weight in pounds.	Remarks.
Mar. 20.....	0.10	69	13	
Mar. 28.....	—	—		Adrenal extirpation.
Mar. 29.....	0.10	71		Good recovery.
Apr. 8.....	0.10	74	13	
Apr. 25.....				Sympathetic to adrenal fragment cut.
Apr. 26.....	0.10	59	13.3	Good recovery.
May 3.....	0.10	72	13.5	
June 11.....	—	—	—	Autopsy.

ADRENAL EXTIRPATION.

Dog A–5.—Black mongrel pup, female.

Dec. 8, 1913. Ether anesthesia and operation.[8] Right adrenal removed completely. Left adrenal in large part removed leaving small fragment, estimated at about one fifth of the gland.

Dec. 9, 12 M. Dog in fair condition; weight 10 pounds. Phthalein 0.09 gm. intravenously.

Dec. 10. Soft feces. Phthalein excretion 17 per cent.

Dec. 16, 1 P.M. Dog greatly improved and quite active, in spite of some loss of weight (9 pounds). Phthalein 0.1 gm. intravenously.

Dec. 17. Soft feces. Phthalein excretion 24 per cent.

Dec. 18. Much fluid feces. Phthalein negative. Dog appears prostrated. Pulse is very weak. Temperature 33.5° C. 4 P.M. Death.

Autopsy.—Gastritis, which may have been associated with purgation. Other organs normal. Adrenal fragment had undergone the usual hypertrophy. Liver and bile passages are quite normal.

ADRENAL EXTIRPATION.

Dog 13–107.—Strong mongrel pup, female; weight 19¼ pounds.

Apr. 8, 1 P.M. Phthalein 0.2 gm. intravenously.

Apr. 9. Abundant feces. Phthalein excretion 74 per cent.

Apr. 20. Ether anesthesia and operation. Right adrenal extirpated with the

[8] This operation was performed by Dr. S. J. Crowe.

exception of a tiny bit of parenchyma at the upper pole, less than one tenth of the substance of the gland. Blood supply much interfered with. Left adrenal extirpated leaving the lower one fourth *in situ*. The blood supply to this fragment was also somewhat interfered with. A good deal of bleeding and handling of viscera.

Apr. 21. Dog looks well, but pulse is weak and rapid. Diarrhea present. Temperature 37.5° C.

Apr. 22, 12 M. Dog in fair condition; weight 18 pounds. Temperature 38.2° C. Phthalein 0.2 gm. intravenously.

Apr. 23. Fluid feces. Phthalein excretion 28 per cent. Dog refuses food and given milk by stomach tube. 3 P.M. Death.

Autopsy.—Performed at once. The peritoneal cavity shows a little peritonitis at the site of operation, consisting mostly of fibrin, and there is no free fluid. Adrenal fragments show considerable edema and obvious necrosis, but some parenchyma seems normal. Liver and bile passages are normal. In this animal the adrenal insufficiency was close to the lowest possible limit.

The two preceding experiments (dogs A–5 and 13–107) show the remarkable depression of liver function that may be present in dogs that have almost the minimum necessary adrenal parenchyma. Both these dogs were close to this line of minimum adrenal tissue and the last dog might well have survived but for the peritonitis, which was too much for the animal in this condition of grave adrenal insufficiency. Such a drop in liver function to one third normal is found only in severe hepatic injury by poisons which, like chloroform, may destroy one half or more of the liver epithelium. This brings out the paralyzing effect of this adrenal insufficiency upon the liver cells which are quite normal histologically.

PANCREAS EXTIRPATION.

Dog 13–20.—Mongrel pup, male; weight 11½ pounds.

Apr. 19, 11 A.M. Phthalein 0.1 gm. intravenously.

Apr. 20. Phthalein excretion 59 per cent.

Apr. 23, 12 M. Ether anesthesia and operation. Pancreas completely extirpated, with clean dissection along the duodenum.

Apr. 24. Pup is active and lively. Weight 10¾ pounds. Phthalein 0.1 gm. intravenously.

Apr. 25. Abundant feces. Phthalein excretion 45 per cent.

Apr. 26, 11 A.M. Dog in good condition; weight 10½ pounds. Phthalein 0.1 gm. intravenously. This caused no appearance of phthalein in the urine.

Apr. 27. Abundant, soft feces. Phthalein excretion 24 per cent.

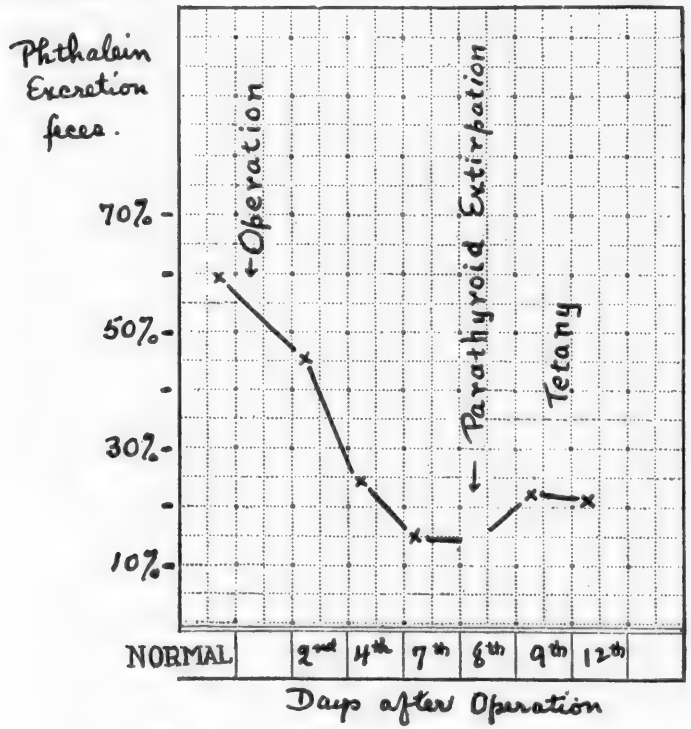
Apr. 28. Dog rather drowsy and eats but little.

Apr. 29. Urine contains a large amount of sugar. Dog is losing weight steadily (9 pounds). 11 A.M. Phthalein 0.1 gm. intravenously. 4 P.M. Urine contains phthalein 3.5 per cent.

Apr. 30. Much feces with diarrhea. Phthalein excretion 15 per cent. 3 P.M. Ether anesthesia for twenty minutes. Thyroid and parathyroid glands removed completely.

May 1. Dog refused food and given milk by stomach tube. Feces are fluid and slightly blood tinged. There is definite evidence of tetany, muscular twitchings being in evidence in the neck. 11 A.M. Phthalein 0.1 gm. intravenously.

May 2. Dog is dull and drowsy. Small amount of feces. Phthalein content 22 per cent.



TEXT-FIG. 2. Dog 13–20. Pancreas extirpation.

May 4, 9 A.M. Dog in poor condition. Temperature 39.5° C. Phthalein 0.1 gm. intravenously.

May 5, 9 A.M. Dog found dead. Some fluid feces in cage containing 17 per cent. phthalein. Urine contained no phthalein but some bile pigment.

Autopsy.—Performed at once. Intestinal contents as well as gall bladder analyzed for phthalein. 4 per cent. found, making a total phthalein output in about twenty hours of 21 per cent., which is about equal to or slightly more than

that of May 2. Lungs show a little bronchopneumonia in lower lobe. Adrenals show a slight atrophy of the cortex. Spleen is rather small, because of interference with the vascular supply at the time of operation on the pancreas. The liver is very fatty, suggesting the picture of phosphorus poisoning.

Microscopical Examination.—Liver cells about the portal spaces for a distance of one or two rows appear normal. Those in the center of the lobule contain great numbers of small fat droplets, but the nuclei are well preserved. No cell necrosis and no wandering cells in the capillaries. Adrenal cells contain a great amount of fat, but appear normal (text-figure 2).

PANCREAS EXTIRPATION.

Dog 13-98.—Yellow adult, male; weight 13½ pounds.

Mar. 19. Ether anesthesia and operation, with complete removal of pancreas, except a few small lobules close to the duodenum.

Mar. 20. Dog appears well. 12 M. Phthalein 0.1 gm. intravenously.

Mar. 21. Urine contains sugar, but no phthalein.

Mar. 22. Abundant feces, phthalein excretion 58 per cent. 12 M. Dog in good condition; weight 12½ pounds. Phthalein 0.1 gm. intravenously.

Mar. 23. Catheterized urine contains no phthalein but considerable sugar.

Mar. 24. Much feces. Total phthalein excretion 56 per cent. 4 P.M. Phthalein 0.1 gm. intravenously.

Mar. 25. Abundant fluid feces. Phthalein excretion 65 per cent.

Mar. 28. Condition unchanged; weight 13 pounds. 3 P.M. Phthalein 0.1 gm. intravenously.

Mar. 29. Abundant feces. Phthalein excretion 46 per cent.

Apr. 16. Dog in poor condition and much emaciated; weight 8½ pounds. 11 A.M. Phthalein 0.1 gm. intravenously. 4 P.M. Urine contains phthalein 1.2 per cent.

Apr. 17. Abundant feces. Phthalein excretion 44 per cent.

Apr. 18, 12 M. Ether anesthesia and operation with complete removal of thyroid and parathyroid glands. Anesthesia twenty minutes.

Apr. 19, 11 A.M. Dog shows signs of tetany; weight 9 pounds. Phthalein 0.1 gm. intravenously.

Apr. 20. Abundant feces. Phthalein output 50 per cent. Typical signs of tetany present.

Apr. 21, 12 M. Condition remains the same; weight 9 pounds. Temperature 37.2° C. Phthalein 0.1 gm. intravenously. 4 P.M. Urine contains phthalein 1.9 per cent.

Apr. 22. Dog in violent tetany. Abundant soapy feces. Phthalein excretion 55 per cent. 4 P.M. Death.

Autopsy.—Performed at once. Thorax, heart, and lungs negative. Total absence of subcutaneous and visceral fat. Kidneys normal. Adrenals are rather small, with atrophy of the cortex. Pancreas: there are a few tiny beads of parenchyma close to the duodenum and pylorus. The liver shows no evidence of fat. It looks rather atrophic. The lobules are regular and normal in color.

Microscopical Examination.—The adrenal glomerular zone is narrow. Fat

is inconspicuous in the cells. The liver shows slight central congestion. The liver cells show distinct atrophy, somewhat similar to that seen with the Eck fistula. Fatty change is not conspicuous. Phagocytic endothelial cells containing pigment are present in the capillaries.

DOG 13–98.

Pancreas Extirpation.

Date.	Phthalein in gm.	Phthalein excretion in per cent. in feces.	Weight in pounds.	Remarks.
Mar. 19.....	—	—	—	Pancreas extirpation.
Mar. 20.....	0.10	58	13.5	
Mar. 22.....	0.10	56	12.5	Sugar in urine.
Mar. 23.....	0.10	65	13.5	
Mar. 28.....	0.10	46	13.0	
Apr. 16.....	0.10	44	8.5	Dog emaciated and weak.
Apr. 18.....	—	—	—	Thyroid, parathyroid extirpation.
Apr. 19.....	0.10	50	9.0	Tetany present.
Apr. 21	0.10	55	9.0	Tetany marked.
Apr. 22.....	—	—	—	Death.

The two preceding experiments (dogs 13–20 and 13–98) show the same drop in phthalein output after pancreatic extirpation. The fall in the phthalein curve is much more marked when the pancreas removal is complete. Given this fall in phthalein curve due to pancreatic insufficiency, the onset of parathyroid tetany will cause a distinct rise in phthalein excretion. This experiment gives better evidence for the acceleration of liver function during parathyroid tetany than can be gotten in simple cases of tetany which will be discussed later.

PANCREAS EXTIRPATION.

Dog 13–104.—Mongrel black and tan, male.

Mar. 25, 4 P.M. Ether anesthesia and operation. Pancreas removed almost completely. A few tiny bits remain close to the duodenum.

Mar. 26. Dog in good condition.

Mar. 27. Dog doing well. Urine contains much sugar. Weight 14¼ pounds.

Mar. 28. Sugar abundant in urine. Weight 13 pounds. 3 P.M. Phthalein 0.1 gm. intravenously.

Mar. 29. Abundant soft feces. Phthalein excretion 47 per cent.

Apr. 2. Phthalein 0.1 gm. intravenously.

Apr. 3. Abundant feces. Phthalein output 56 per cent.

Apr. 8, 1 P.M. Dog is weak and emaciated. Weight 9½ pounds. 12 M. Phthalein 0.1 gm. intravenously. 6 P.M. Urine contains phthalein 2 per cent.

Apr. 9. Abundant feces, phthalein excretion 32 per cent.

Apr. 10, 2 P.M. Dog in poor condition and very weak. Ether anesthesia and bleeding.

Autopsy.—Performed at once. Thorax, heart, and lungs normal. Fat has completely disappeared. All viscera are normal except the pancreas and liver. One tiny nodule of the pancreas close to the duodenum remains. Dimensions 3 by 5 by 1 mm. Liver shows some focal yellow areas and the centers of all lobules are fatty. Elsewhere the parenchyma is brownish and translucent.

Microscopical Examination.—Liver resembles closely that of dog 13–98. The cells show a little atrophy and slight fatty degeneration. Large pigmented endothelial cells are found in the blood vessels. There are a few polymorphonuclears in the capillaries. No necroses.

THYROID AND PARATHYROID EXTIRPATION.

Dog 13–51.—Mongrel adult, male; weight 15½ pounds.

Jan. 17, 12 M. Ether anesthesia and operation; thyroid and parathyroids removed. At the end of the operation intravenous injection of phthalein 0.1 gm.

Jan. 18. Good recovery.

Jan. 19. Muscular tremors definite. Abundant feces. Phthalein output 35 per cent. 12 M. Muscular tremors are more marked; weight 16¾ pounds. Phthalein 0.1 gm. intravenously. 5 P.M. Convulsive seizures.

Jan. 20 and 21. Tremors present but less violent with milk diet. Total phthalein output 60 per cent.

Jan. 21, 6 P.M. Phthalein 0.1 gm. intravenously.

Jan. 23, 4 P.M. Total phthalein output 46 per cent. Delay in purgation, which was incomplete until Jan. 24, may explain this in part.

Jan. 24, 6 P.M. Phthalein 0.1 gm. intravenously. Weight 15½ pounds. Dog on milk diet.

Jan. 26. No feces.

Jan. 27. Much diarrhea. Total phthalein output 57 per cent. Dog in violent tetany and the slightest touch causes a violent spasmodic reaction. 5 P.M. Death.

Autopsy.—Performed at once. The autopsy showed nothing abnormal except venous engorgement of organs.

Microscopical Examination.—Liver normal.

THYROID AND PARATHYROID EXTIRPATION.

Dog 13–13.—Fox-terrier, male; weight 15¾ pounds.

Nov. 13, 12 M. Phthalein 0.08 gm. intravenously.

Nov. 15. Abundant feces. Phthalein output 45 per cent.

Dec. 23, 12 M. Dog in good condition. Ether anesthesia, and operation. Thyroid and parathyroids removed.

Dec. 24. Muscular tremors are present; weight 10½ pounds. 1 P.M. Phthalein 0.1 gm. intravenously.

Dec. 25. Abundant feces. Phthalein excretion 42 per cent.

Dec. 26. Phthalein 0.1 gm. intravenously.

Dec. 27. Muscular tremors very marked. Dog refuses food; weight 11½ pounds. Abundant feces. Phthalein output 68 per cent.

Dec. 28. Death.
Autopsy.—Normal organs throughout.

The two preceding experiments give definite evidence that there is no decrease in liver functional capacity even during the extreme prostration of fatal tetany. The last of the series (dog 13–13) suggests a definite rise above the normal base line. It must be recalled that the feces excretion of phenoltetrachlorphthalein may be very nearly the same whether the drug is injected intravenously or given by stomach tube. This shows how completely the drug is excreted by the normal liver and how little is lost in the transfer from the blood to the bile. One experiment may be cited as illustrative of this point.

Dog 13–125.—Young female pup; weight 14½ pounds.
May 6, 4 P.M. Phthalein 0.1 gm. intravenously.
May 8 and 9. Total phthalein excretion 57 per cent.
May 8, 12 M. Phthalein 0.1 gm. given with milk by stomach tube.
May 9, 12 M. Phthalein excretion 60 per cent.

This experiment shows how narrow the margin may be between the normal phthalein excretion and presumably total excretion into the alimentary tract. Other experiments may show a much greater difference.

One of the experiments given below (dog 13–36) gives evidence of a slight rise in phthalein excretion above normal in periods of mild tetany, as it is clear that this dog suffered at times from parathyroid insufficiency and died in tetany. Better and more convincing evidence that tetany causes an increase in phthalein liver excretion is found in the preceding experiments under pancreatic extirpation (dog 13–20 (text-figure 2) and dog 13–98). With a falling phthalein curve due to pancreatic insufficiency we see a definite rise caused by parathyroid tetany. This rise is noted, although the general condition of the dog is much less favorable than before the removal of the thyroid and parathyroid glands.

From all this data it seems safe to conclude that during tetany there is a tendency towards an increase in phthalein excretion by the liver, indicating an overstimulus and hyperactivity of the liver epithelium.

THYROID EXTIRPATION.

Dog 13–26.—Active adult, male; weight 20½ pounds.

Jan. 26, 3 P.M. Phthalein 0.2 gm. intravenously.

Jan. 27. Abundant feces. Phthalein output 65 per cent.

Mar. 21, 11 A.M. Operation with ether anesthesia. Extirpation of both thyroid lobes, one large parathyroid being left at the lower pole on the left side.

Mar. 22. Dog is well; weight 19 pounds. 12 M. Phthalein 0.2 gm. intravenously.

Mar. 23. Dog is normal. No feces.

Mar. 24. No signs of tetany. Abundant feces. Total output of phthalein 63 per cent.

Mar. 25. No muscular tremors. Weight 19¼ pounds. Appetite good. 12 M. Phthalein 0.2 gm. intravenously.

Mar. 26. Abundant feces. Phthalein excretion 63 per cent.

Apr. 16, 11 A.M. Dog in normal condition; weight 18¾ pounds. Phthalein 0.2 gm. intravenously.

Apr. 17. Abundant feces. Phthalein output 66 per cent.

June 8. Dog has been in good condition over the entire period since the operation. Weight 20½ pounds. 12 M. Phthalein 0.2 gm. intravenously.

June 9. Abundant feces. Phthalein excretion 67 per cent.

June 11. Ether anesthesia and bleeding.

Autopsy.—Performed at once. All the organs are perfectly normal. Neck shows no definite thyroid tissue. One large parathyroid is found and appears to be normal. Liver and bile passages normal.

Microscopical Examination.—Liver normal. Two tiny bits of thyroid tissue found in section. The acini contain little colloid and show a cubical type of epithelium. Parathyroid large and normal. Other organs normal.

DOG 13–26.

Thyroid Extirpation.

Date.	Phthalein in gm.	Phthalein excretion per cent. in feces.	Weight in pounds.	Remarks.
Jan. 26.....	0.20	65	—	
Mar. 21.....				Thyroid extirpation.
Mar. 22.....	0.20	63	19.0	No tetany.
Mar. 25.....	0.20	63	19.3	No tetany.
Apr. 16.....	0.20	66	18.7	No tetany.
June 8.....	0.20	67	20.5	Good condition.
June 11.....	—	—	—	Autopsy.

THYROID EXTIRPATION.

Dog 13–36.—Active young female; weight 21 pounds.

Jan. 19, 12 M. Phthalein 0.15 gm. intravenously.

Jan. 20. Abundant feces. Phthalein output 56 per cent.

Jan. 23. Chloroform 15 c.c. by stomach tube, causing slight intoxication.

Jan. 24. Dog will eat little food; weight 20¾ pounds. 6 P.M. Phthalein 0.2 gm. intravenously.

Jan. 26. Abundant feces. Total phthalein output 19 per cent.

Jan. 28. Condition appears to be normal.

Feb. 7, 11 A.M. Ether anesthesia with removal of thyroid lobes. One parathyroid at left upper pole was left undisturbed.

Feb. 8. Dog appears well, except for slight muscular tremors. 10 A.M. Phthalein 0.2 gm. intravenously.

Feb. 9. Abundant feces. Total phthalein output 70 per cent.

Feb. 12. Dog active and hungry.

Feb. 15. Dog in good condition; weight 22 pounds. 11 A.M. Phthalein 0.2 gm. intravenously.

Feb. 16, 12 M. Abundant feces. Phthalein excretion 66 per cent.

Mar. 18. Dog in good condition, except for a little mange. Weight 21½ pounds. 4 P.M. Phthalein 0.2 gm. intravenously.

Mar. 19. Abundant feces. Phthalein output 52 per cent.

Apr. 16, 11 A.M. Dog in good condition. Weight 19¾ pounds. Phthalein 0.2 gm. intravenously.

Apr. 17. Soft feces. Phthalein output 62 per cent.

May 6, 4 P.M. Dog in good condition. Weight 21½ pounds. Phthalein 0.2 gm. intravenously.

May 7. No feces.

May 8. Abundant feces. Phthalein output 53 per cent.

June 1. Dog has developed violent tetany which resists all treatment.

June 2. Death.

Autopsy.—Organs showed advanced postmortem change.

DOG 13–36.

Thyroid and Partial Parathyroid Extirpation.

Date.	Phthalein in gm.	Phthalein excretion per cent. in feces.	Weight in pounds.	Remarks.
Jan. 20.....	0.15	57	21.0	
Jan. 23.....	—	—	—	Chloroform by stomach (15 c.c.)
Jan. 24.....	0.20	19	20.7	
Feb. 7.....	—	—	—	Thyroid and 3 parathyroids extirpated.
Feb. 8.....	0.20	70	18.5	Slight tetany.
Feb. 15.....	0.20	67	22.0	Good condition.
Mar. 18.....	0.20	53	21.5	
Apr. 16.....	0.20	62	19.7	
May 6.....	0.20	53	21.5	Good condition. No tetany.
June 1.....	—	—	—	Violent tetany. Death.

The two preceding experiments (dogs 13–26 and 13–36) show that thyroid insufficiency has no effect upon the curve of phthalein liver excretion. During periods of tetany due to removal of too much parathyroid tissue the excretion curve may fluctuate. It is possible that longer periods of observation may bring out a change

in hepatic function with complete thyroid removal. Observations on human cases of exophthalmic goitre would be of interest in this connection.

HYPOPHYSIS EXTIRPATION.

Dog 13–79.—Active male pup; weight 15 pounds.

Feb. 18, 11 A.M. Phthalein 0.1 gm. intravenously.

Feb. 19. Abundant feces. Phthalein excretion 70 per cent.

Mar. 20, 12 M. Dog has practically recovered from distemper; weight 12½ pounds. Phthalein 0.1 gm. intravenously.

Mar. 22. Delay in purgation. Phthalein output 63 per cent.

Apr. 20. Ether anesthesia and operation.[9] Removal of hypophysis.

Apr. 21. Dog in good condition. Temperature 39.2° C. 12 M. Phthalein 0.1 gm. intravenously. Weight 15½ pounds.

Apr. 22. No feces.

Apr. 23. Soft feces. Phthalein output 52 per cent. Edema of head wound is marked. Temperature 39.2° C.

DOG 13–79.

Hypophysis Extirpation.

Date.	Phthalein in gm.	Phthalein excretion per cent. in feces.	Weight in pounds.	Remarks.
Feb. 18.....	0.10	70	15.0	Young pup.
Mar. 20.....	0.10	63	12.5	Recovering from distemper.
Apr. 20.....	—	—	—	Hypophysis extirpation (incomplete).
Apr. 21.....	0.10	52	15.5	Slight fever.
Apr. 24.....	0.10	57	15.0	Improving.
Apr. 27.....	0.10	67	14.7	Good condition.
Apr. 29.....	0.10	76	15.0	
May 3.....	0.11	57	15.0	Good condition.
May 8.....	0.10	52	16.5	
June 8.....	0.10	61	16.5	Good condition.
June 11.....	—	—	—	Autopsy. Remnant of anterior lobe.

Apr. 24, 12 M. Dog appears normal; weight 15 pounds. Phthalein 0.1 gm. intravenously.

Apr. 25. Abundant feces. Phthalein output 57 per cent.

Apr. 27, 12 M. Condition unchanged; weight 14¾ pounds. Phthalein 0.1 gm. intravenously.

Apr. 28. Abundant feces. Phthalein output 67 per cent.

Apr. 29. Condition normal. 11 A.M. Phthalein 0.1 gm. intravenously.

Apr. 30. Abundant feces. Phthalein output 76 per cent.

May 3. Condition the same; weight 15 pounds. 12 M. Phthalein 0.11 gm.

May 4. Abundant feces. Phthalein output 57 per cent.

May 8. Good condition; weight 16½ pounds. 12 M. Phthalein 0.1 gm. intravenously.

[9] This operation was performed by Dr. A. P. Jones.

May 9. Abundant feces. Phthalein output 52 per cent.

June 8, 12 M. Good condition; weight 16½ pounds. Phthalein 0.1 gm. intravenously.

June 9. Abundant feces. Phthalein output 61 per cent.

June 11. Ether anesthesia and bleeding.

Autopsy.—Performed at once. Hypophysis absent except a small fragment of anterior lobe at the base of the sella turcica. All other organs appear normal in all respects. The subcutaneous fat is well preserved. Liver and bile passages are normal.

Microscopical Examination.—Liver is normal. Other organs are negative.

HYPOPHYSIS EXTIRPATION.

Dog 13–74.—Active young male; weight 18½ pounds.

Feb. 10, 10 A.M. Phthalein 0.1 gm. intravenously.

Feb. 11. Abundant feces. Phthalein output 66 per cent.

Feb. 17. Ether anesthesia and operation.[10] Complete removal of hypophysis.

Feb. 18. Condition good. 11 A.M. Phthalein 0.1 gm. intravenously.

Feb. 19. No feces.

Feb. 20. Abundant feces. Phthalein output 55 per cent.

Feb. 21. Dog improving; weight 20¼ pounds. 12 M. Phthalein 0.1 gm. intravenously.

Feb. 23. Abundant feces. Phthalein output 65 per cent.

Feb. 25, 11 A.M. Dog in good condition; weight 18½ pounds. Phthalein 0.1 gm. intravenously.

Feb. 26. Abundant feces. Phthalein output 69 per cent.

Feb. 28. Convulsions and coma, with almost complete absence of pulse, giving the appearance of death.

Mar. 1, 10 A.M. Dog is quite toxic. Temperature 36.8° C. Weight 17 pounds. Phthalein 0.1 gm. intravenously.

Mar. 2. Dog in semistupor. Temperature 36.7° C. No feces.

Mar. 3, 9.30 A.M. Pulse slow. Temperature 31.3° C. Weight 16 pounds. Thin, blood stained feces. Phthalein excretion 50 per cent. 2 P.M. Death.

DOG 13–74.

Hypophysis Extirpation.

Date.	Phthalein in gm.	Phthalein excretion per cent. in feces.	Weight in pounds.	Remarks.
Feb. 10.....	0.10	66	18.5	
Feb. 17.....	—	—	—	Hypophysis extirpated.
Feb. 18.....	0.10	55	18.7	
Feb. 21.....	0.10	65	20.3	Dog improving.
Feb. 25.....	0.10	69	18.5	Good condition.
Feb. 28.....	—	—	—	Convulsions and coma.
Mar. 1.....	0.10	50	17.0	Subnormal temperature.
Mar. 3.....	—	—	—	Death. Temperature 31.3° C.

[10] This operation was performed by Dr. S. J. Crowe.

Autopsy.—Performed at once. All organs are normal. Liver and bile passages are normal.

Microscopical Examination.—Spleen and lymph glands show slight hyperplasia of lymph follicles. Liver shows acute engorgement of centers of lobules.

HYPOPHYSIS EXTIRPATION.

Dog H–3.—Mongrel female; weight 17 pounds.

Jan. 26. Ether anesthesia and operation.[11] Complete removal of hypophysis.

Jan. 28. Dog in good condition; weight 17¼ pounds. 12 M. Phthalein 0.1 gm. intravenously.

Jan. 29. Abundant feces. Phthalein 41 per cent.

Jan. 31. Dog looks sick. Temperature 39.6° C. Weight 16½ pounds. Phthalein 0.1 gm. intravenously.

Feb. 1. Abundant feces. Phthalein output 62 per cent.

Feb. 3. Condition fair; weight 16 pounds. Temperature 39.8° C. Phthalein 0.1 gm. intravenously.

Feb. 4. Abundant feces. Total phthalein output 57 per cent.

Feb. 7. Dog is drowsy. 3 P.M. Phthalein 0.1 gm. intravenously. Weight 17¼ pounds.

Feb. 8. Abundant feces. Phthalein output 47 per cent.

Feb. 10. Dog developed definite signs of distemper.

Feb. 12. Dog is drowsy; weight 15½ pounds. 5 P.M. Phthalein 0.1 gm. intravenously.

Feb. 13. No feces.

Feb. 14. Abundant feces. Phthalein excretion 46 per cent.

DOG H–3.

Hypophysis Extirpation.

Date.	Phthalein in gm.	Phthalein excretion per cent. in feces.	Weight in pounds.	Remarks.
Jan. 26.....				Hypophysis removal.
Jan. 28.....	0.10	41	17.3	Temperature 39.4° C.
Jan. 31.....	0.10	62	16.5	Temperature 39.6° C.
Feb. 3.....	0.10	57	16.0	
Feb. 7.....	0.10	47	17.3	Dog very drowsy.
Feb. 12.....	0.10	46	15.5	Distemper mild.
Feb. 16.....	0.10	40	14.0	Convulsions.
Feb. 17.....	—	—	—	Death.

Feb. 16. Dog in convulsions. Temperature 40.9° C. Weight 14 pounds. 3.30 P.M. Phthalein 0.1 gm. intravenously.

Feb. 17. Dog in stupor; pulse weak. 10.30 A.M. Death. Phthalein in cage and from intestinal contents combined, 40 per cent.

Autopsy.—Performed at once. Lungs show ecchymoses and some small patches of bronchopneumonia. Pancreas shows fat necroses with hemorrhage,

[11] This operation was performed by Dr. A. P. Jones.

an example of spontaneous hemorrhagic pancreatitis. Kidneys show small gray nodules in the cortex. Intestinal tract negative. Liver is rather pale and fatty.

Microscopical Examination.—Pancreatitis is of considerable age, showing a good deal of scar tissue throughout the gland, although there are some relatively fresh necroses of pancreas parenchyma and fat. The lungs show atelectasis and some bronchopneumonia. The kidneys show accumulations of mononuclear wandering cells in some parts of the cortex. Parenchyma is normal. There is slight atrophy of the liver cells in the center of the lobule. Otherwise normal.

The three preceding experiments are uniform in all respects. Complete removal of the hypophysis will cause little change in the liver function, as indicated by phthalein excretion. There is an initial drop in the phthalein excretion following the operation, and this cannot be attributed to the anesthetic. Possibly the cerebral edema and trauma may account for it. This is followed by a recovery to normal or even a little above normal. Just before the fall in body temperature, which usually precedes death in these dogs, there is a definite impairment of liver function. In the experiment (dog 13–79) in which a bit of the anterior lobe remained, one sees minor fluctuations in the phthalein curve, which may be dependent in part on the hypophysis insufficiency which was undoubtedly present.

SUMMARY.

When phenoltetrachlorphthalein is injected intravenously, it is eliminated from the body in the bile through the activity of the hepatic epithelium. The feces may be collected after purgation and the phthalein extracted and estimated against a standard solution. The estimation of phthalein can be done with accuracy in a suitable colorimeter and the elimination in normal dogs is quite constant.

Given a definite liver injury by means of poisons (chloroform, phosphorus), the amount of phthalein excreted will be diminished and the fall in output will be proportional to the amount of injury. With an acute fatal poisoning the curve may fall to zero.

Under certain conditions of vascular interference the liver phthalein may show a decreased output; in passive congestion of the liver and with the Eck fistula the liver output may fall considerably below normal.

Known disturbances of the liver function due to parenchymatous

injury or vascular disturbances are indicated by a fall in the phthalein excretion curve. Conversely it may be claimed that a drop in phthalein excretion may indicate a decrease in the functional capacity of the liver even if there be no detectable histological changes.

Adrenal insufficiency produced by extirpation of three fourths or more of the gland tissue will be associated with a drop in liver phthalein excretion. With hypertrophy of the adrenal fragment the excretion comes back to normal, but may fall again when more adrenal tissue is removed (text-figure 1).

Pancreatic insufficiency causes a progressive fall in the phthalein excretion indicating a grave lowering of the functional capacity of the liver (text-figure 2). This fact has a direct bearing on the question of diabetes.

Parathyroid insufficiency with tetany causes no decrease in phthalein output, but at times a rise above normal. This comes out best when the phthalein curve is low following pancreas extirpation. Parathyroid tetany may cause hyperactivity on the part of the liver cells.

Thyroid insufficiency produces no change in the uniform curve of phthalein excretion.

Hypophysis insufficiency shows an initial fall in the curve, followed by a return to normal and a final drop in the last few days before death.

These experiments supply evidence to the effect that the liver is very much concerned in the derangement that follows the removal of the ductless glands. Hence it seems probable that this disturbance of the liver function may be an important factor in the general symptom complex of ductless gland insufficiency.

In conclusion we wish to express our appreciation to Dr. S. J. Crowe and Dr. A. P. Jones for assistance rendered in performing some of the operations for gland removal.